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A Summary of Current Program and Preliminary Report of Progress

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BEEF CATTLE RESEARCH

of the

United States Department of Agriculture and Cooperating Agencies

This progress report of U.S.D.A. and cooperative research is primarily a tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on U.S.D.A. and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having an interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued during the last year. Current agricultural research findings are also published in the monthly U.S.D.A. publications, Agricultural Research, Agricultural Marketing, and The Farm Index.

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D. C.

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ADVISORY COMMITTEES

The research program of the Department of Agriculture is reviewed annually by the following advisory committees: by the following advisory committees:

- l. Farm Resources Research
- Utilization Research and Development 2.
- Human Nutrition and Consumer Use Research
- Marketing Research and Service
- Agricultural Economics Research
- 6. Forestry Research
- 7. Animal and Animal Products Research
- Cotton and Tobacco Research
- 9. Grain and Forage Crops Research 10. Horticultural Crops Research
- 11. Oilseed, Peanut and Sugar Crops Research

ORGANIZATIONAL UNIT PROGRESS REPORTS

The source materials used by the advisory committees are of two types. First, there are Organizational Unit Reports that cover the work of the Divisions or Services listed below. The number prefixes refer to advisory committees listed above that review all of the work of the respective Divisions or Services.

Agricultural Research Service (ARS)

- 1 Soil and Water Conservation
- 2 Utilization -- Eastern
- 2 Utilization -- Northern
- 2 Utilization -- Southern
- 2 Utilization -- Western
- 3 Human Nutrition
- 3 Clothing and Housing
- 3 Consumer and Food Economics
- 7 Animal Husbandry
- 7 Animal Disease and Parasite

Agricultural Marketing Service (AMS)

- 4 Market Quality
- 4 Transportation and Facilities

Economic Research Service (ERS)

- 4,5 Marketing Economics
- 5 Farm Production Economics
- 5 Resource Development Economics
- 5 Economic & Statistical Analysis
- 5 Foreign Development and Trade Analysis
- 5 Foreign Analysis Division

Other Services

- 1 Soil Conservation Service (SCS)
- 4,5 Farmer Cooperative Service (FCS)
- 4,5 Statistical Reporting Service (SRS)
- 6 Forest Service (FS)

Three organizational unit reports are not reviewed in entirety by any one committee. All of the information in them is included in the subject matter reports.

Agricultural Research Service (ARS)

Agricultural Engineering Crops Entomology

SUBJECT MATTER PROGRESS REPORTS

The second type of report brings together the U.S.D.A. program and progress for the following commodities and subjects:

- 1 Cross Commodity Research of Agricultural Engineering, Crops, & Entomology Research Divisions
- 3 Rural Dwellings
- 6 Forestry (Other than Forest Service)
- 7 Beef Cattle
- 7 Dairy 7 - Poultry
- 7 Sheep and Wool
- 7 Swine
- 7 Cross Specie & Miscellaneous Animal Research

- 8 Cotton and Cottonseed
- 8 Tobacco
- 9 Grain and Forage Crops
- 10 Citrus & Subtropical Fruit
- 10 Deciduous Fruit & Tree Nut
- 10 Potato
- 10 Vegetable
- 10 Florist, Nursery & Shade Tree
- 11 Oilseed and Peanut
- 11 Sugar

A copy of any of the reports may be requested from Max Hinds, Executive Secretary, Animal and Animal Products Research Advisory Committee, Agricultural Research Service, U. S. Department of Agriculture, Washington 25, D. C.

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INTRODUCTION

This report on beef research covers work directly related to the production, processing, distribution, and consumption of beef cattle and resulting products. The information has been assembled from the organizational unit reports of the several divisions. This report does not include extensive cross-commodity work, much of which is basic in character, which contributes to the solution of not only beef problems but also to the problems of other commodities. Progress on cross-commodity work is found in the reports of the several divisions such as Soil and Water Conservation, Human Nutrition, Transportation and Facilities, Farm Production Economics, Foreign Development and Trade Analysis, and Cross-Specie and Miscellaneous Animal Research.

This report is devoted to the 13 "problem areas" shown in the table of contents. For each area there is a statement of (1) the Problem, (2) the USDA Program, (3) A summary of Progress during the past year on USDA and cooperative work, and (4) A list of Publications resulting from USDA and cooperative work.

Beef research can be divided into three major categories, i.e., that supported by (1) Federal funds appropriated to the research agencies of the United States Department of Agriculture, (2) Federal and State funds appropriated to the 53 State Agricultural Experiment Stations, and (3) private funds allotted, largely by the beef industry, to research carried on in private laboratories or to support of State Station or USDA work. For all three categories it is estimated that about 1,300 scientists are engaged in research dealing specifically with the production, processing, distribution, and consumption of beef and its products. Support of their work involves an annual expenditure of between 30 and 35 million dollars. This amounts to 0.6% of the cash farm receipts from the sale of beef cattle and 0.4% of the retail cost of beef. Of the 1300 scientists engaged in beef research, approximately 19% are employed by the Department of Agriculture, 20% by the State Experiment Stations, and 61% by other universities, foundations, and private industry.

Research by USDA

Farm research pertaining to beef is conducted in the Agricultural Research Service divisions of Agricultural Engineering, Animal Disease and Parasite, Animal Husbandry, and Entomology. The work comprises investigations of breeding, physiology, nutrition, diseases, insects, housing and management, involving 188 professional man-years of scientific effort. This includes research on cattle diseases and parasites that is applicable to dairy, also.

Nutrition, consumer, and industrial use research pertaining to beef is ducted in the Agricultural Research Service divisions of Human Nutrition, Consumer and Food Economics, and Eastern Utilization. The work comprises

investigations of composition and nutritive value; physiological availability of nutrients and their effects; new and improved methods of preparation, preservation, and care in homes, eating establishments and institutions; and with the processing phase involving slaughtering the animals and processing the meat, tallow and hides. Also, it is concerned with improved equipment and processes. The work in these divisions involves 41 professional man-years of scientific effort.

Marketing and economic research pertaining to beef is carried on within four Services: Agricultural Marketing Service, Economic Research Service, Farmer Cooperative Service, and Statistical Reporting Service. The work comprises (1) physical and biological aspects of assembly, packaging, transporting, storing and distribution; (2) economic aspects of marketing costs, margins and efficiency, market potential, supply and demand, and situation and outlook; (3) cooperative marketing; and (4) consumer acceptance studies. The divisions in which the work is conducted are: Market Quality, AMS: Transportation and Facilities, AMS; Marketing Economics, ERS; Economic and Statistical Analysis, ERS; Marketing Division, FCS; Standards and Research, SRS. The scientific effort involved by these divisions amounts to 22 professional man-years.

Interrelationships among Department, State and Private Research

A large part of the Department's research is cooperative with State Experiment Stations. Many Department employees are located at State Stations and use laboratory and office space close to or furnished by the Station. Cooperative work is jointly planned, frequently with the participation of representatives of the producers or industry affected. The nature of cooperation varies with each study. It is developed so as to fully utilize the personnel and other resources of the cooperators which frequently includes resources contributed by the interested producers or industry.

Including both cooperative and State Station projects beef research is carried on in 47 of the 53 State Experiment Stations. The types of work to which the largest amount of effort is devoted includes breeding, physiology, nutrition and management, diseases and parasites, marketing economics, and utilization research on meat and animal fats. There is regular exchange of information between Station and Department scientists to assure that the programs complement each other and to eliminate unnecessary duplication.

Privately supported beef research emphasizes the solution of scientific production, processing, and marketing problems. Much of it utilizes the results of basic work done by State Station and Department scientists.

About 1/3 of industry's contribution to the research effort pertains to farm research. In contrast with the poultry industry where practically breeding research is done by industry, very little is done by industry in beef, except the work of large firms like the King Ranch which developed the Santa Gertrudis breed. The scope of operation required for success ful breeding of beef cattle, because of the size of animal and length of life cycle which tie up a substantial amount of funds, is undoubtedly a factor contributing to the amount of public research.

About equal to the farm research effort in the livestock industry, another one-third is in the utilization field. In contrast with the public research in basic work the industry program places strong emphasis on developmental activities and solving of immediate problems. The work of meatpackers is devoted to finding industrial utilization of by-products, quality control devices, improved formulation of products, improved handling and plant arrangement. Independent laboratories and foundations take on short time problem-solving for clients in the meat industry. Pharmaceutical firms carry on research on extraction of biologically active substances from meat by-products such as hormones from glands, and with the development of agents, such as antibiotics for use in meat processing.

The contributions of beef producers and industry to the work of the State Stations and the Department have been an important factor in the success of their research programs. Producers offer herds and facilities for testing products and practices used in production. Likewise, processors and retailers offer facilities and products for use by public research agencies. Many problems in the economics of marketing cannot be transferred to a laboratory, experimental plot, or other simulated situation. The results of economic research conducted cooperatively is of great value to industry, especially in cases where public research can provide comparison and analysis. Even large firms that have a research staff do not have access to the plants and records of competitors.

Examples of Recent Research Accomplishments by USDA and Cooperating Scientists

Ventilation of livestock buildings. Research in cooperation with State

Experiment Stations has obtained much needed basic data on the heat
and moisture given off by cattle, hogs, and poultry, and on the influence of building environment on production and feed consumption. The
heat and moisture dissipation data are considered basic design data
for ventilation systems of poultry, dairy, and swine buildings. They
appear in design handbooks including the 1962 Guide and Data Book of
the American Society of Heating, Refrigeration, Ventilating, and Air
Conditioning Engineers, and are used by makers of ventilating equipment,
prefabricated buildings and package buildings as well as by specialists

advising farmers on their own construction. Building improvements resulting from the above research have contributed to the substantial rise in efficiency of livestock production that has occurred during the past decade.

Microbes help with pesticide residue problems. Ruminal protozoa, cultured as individual species, offer definite promise as a screening technique for determining if pesticides will leave residues in meat and milk of cattle and sheep. Ruminant animals possess large numbers of microorganisms in their digestive system, particularly the rumen which appears to be the natural site for the microbial degradation of complex compounds such as pesticides. Pesticides, if degraded in this manner, will not produce residues in the meat even though they are consumed with the feed. Ruminal protozoa were found to metabolize the following pesticides: Diazinon, dimethoate, lindane, Thiodan, and Sevin.

Emulsifying capacity of meat. A simple technique has been devised by EU scientists to rapidly determine the emulsifying capacity of meat from different sources used in sausage formulations. "Emulsifying capacity" is an important property of meat utilized in manufacture of frankfurters, bologna and other emulsion-cured meat products. This development has facilitated the application of automatic data processing to accurate sausage formulation by providing quantitative measurements of the fat-binding potential of the meat. Use of this technique is proving advantageous to sausage manufacturers from the standpoint of cost and overall product quality.

Biodegradable detergents from tallow. Current emphasis on biodegradability of detergent materials is causing industry to recondiser tallow as a raw material for manufacture of detergents, wetting agents and other surface active agents. Recent investigations by EU chemists indicate that the of-sulfo acids prepared from tallow are biodegradable as measured by the river dye-away test. From recently completed EU research it is known that of-sulfo acids prepared from tallow are effective lime soap dispersing agents. For this reason, they may be expected to be useful in the form of soap-detergent combinations. This research has also shown that certain esters of the of-sulfo acids are especially effective as wetting agents. Since they compare in effectiveness with the best known commercial wetting agents and are also potentially very cheap to manufacture, commercial interest in them seems assured. In the meantime, manufacture of of-sulfo acids and their esters is being undertaken by several commercial concerns.

The influence of bovine age on meat characteristics and grade. The basic study conducted by Oklahoma State University under an AMS contract provided valuable information to evaluate present grade standards for reflecting the effect of age upon meat palatability. It was found that tenderness of the longissimus dorsi steaks as measured by the Warner-Bratzler Shear and panel (with marbling of each carcass at or closely approaching either the "slight amount" or "slightly abundant" level) decreased significantly with increasing animal age. The greatest difference in tenderness was observed between the 18- and 42-month age groups. The effect of aging the meat 14 days varied with animal age, marbling level, and the tenderness measure used. Moisture, ash, and protein contents of loins were not significantly different for the age groups except that the 6-month-old calves had slightly higher moisture values.

Central preparation of meat for retail sale. Exploratory research conducted in small central meat packaging plants indicates that the yearly facility, equipment, and labor costs of processing and packaging meat for retail sales can be reduced as much as 50% in plants with \$250,000 weekly meat volume. This represents a savings equal to 4.6% of retail sales. These findings make a number of assumptions that need to be tested further.



BEEF CATTLE - BREEDING Animal Husbandry Research Division, ARS

Problem. Expression of each of the productive and carcass traits of beef cattle varies from breed to breed and between animals within each breed. The beef cattle producer is constantly striving to achieve excellence in one or more of these traits. Frequently his failure to choose the best animals for breeding stock for the most effective mating program results in less than maximum progress. Often the beef cattle producer does not know how to identify, evaluate and utilize the existing variability to achieve his aim. Research information is needed on heritability of economic traits in beef cattle, genetic and phenotypic correlation between these traits, effectiveness of various selection and breeding programs, and assessment of traits most useful in beef cattle improvement.

USDA PROGRAM

The beef cattle breeding research in the United States has developed as a coordinated program of the USDA and the State Experiment Stations. It is a continuing program of both applied and basic research carried on by geneticists, animal physiologists, and animal husbandmen. Early efforts in the improvement of beef cattle through performance testing were made by the USDA at Miles City, Montana, and Beltsville, Maryland. With the advent of regional research, efforts by the State stations were greatly increased and the individual programs were coordinated through regional research projects in three of the important beef cattle producing regions. This joint activity has been and remains characteristic of beef cattle breeding research, and the resulting program is an integrated effort combining to the best advantage the resources of the State Experiment Stations and the USDA.

The regional project in the South is S-10, Improvement of Beef Cattle for the Southern Region through Breeding Methods. Much of this region is subtropical in climate and in many cases cattle used in other areas are poorly adapted. Environmental conditions adversely affecting survival, reproductive regularity and growth are encountered. Research includes projects at 13 State stations and at the USDA stations at Jeanerette, Louisiana; Front Royal, Virginia; and Brooksville, Florida.

In the Western region the beef industry is largely geared to range conditions with many cattle shipped to areas of abundant grain supply for fattening. Ability to make maximum use of forage available on the range is an important consideration. These problems are studied through regional project W-1, The Improvement of Beef Cattle through the Application of Breeding Methods. Research includes projects at 12 State stations and at the USDA station at Miles City, Montana.

Similarly, NC-1, Improvement of Beef Cattle through Breeding Methods, is geared to problems of the beef industry in the North Central region where beef is produced on farms with pastures of high productivity and ample grain supplies for feedlot finishing. Research includes projects at 12 State stations and at the USDA stations at Fort Robinson, Nebraska, and Fort Reno, Oklahoma.

The <u>Federal</u> scientific effort devoted to research in this area totals 17.5 professional man years. Of this number, 1.3 are devoted to <u>performance</u> testing, 4.7 to genetics and interrelations of performance traits, 1.0 to genetic-environmental interactions, 7.8 to <u>selection</u> and <u>systems</u> of <u>breeding</u>, and 2.7 to <u>program leadership</u>.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Genetics and Interrelationships of Performance Traits

1. Growth rate and efficiency of gain. In the most comprehensive study yet made on the genetic aspects of efficiency of gain (defined as live-weight gain in relation to feed consumed), data on over 1300 animals individually fed showed efficiency of gain to be 62% heritable when adjustments were made for differences in live weight and in feed consumption. Heritability of feed consumption was 64%. Genetic correlations were: growth rate and efficiency of gain, .8; growth rate and feed consumption, .6; and feed consumption and efficiency of gain, zero. This indicates that selection for growth rate will result in improved efficiency of gain with a part of the improvement being due to true improvement in efficiency and part to increased feed consumption. Increased feed consumption increases rate of gain and indirectly increases efficiency of gain through reducing overhead cost of maintenance per unit of gain. (AH dl-12, dl-31)

Several other studies have shown positive genetic relationships between rate and efficiency of gain with the magnitude of the relationship varying considerably between studies. (AH dl-8, dl-22)

The results of these studies, as well as related studies with other species, indicate that selection for high levels of overall productivity is the most potent tool available for increasing net production of the beef industry in relation to feed consumption. However, much more information than now available is needed on variation between individual animals in maintenance requirements.

Data from several stations, including progeny tests of high and low gaining sires, have confirmed previous estimates of high heritability of postweaning growth rate. (AH dl-29, dl-2)

In one study, heritability of growth rate tended to increase as the length of the postweaning evaluation period increased up to 550 days of age. In

general, estimates of heritability tended to be high for growth rate and conformation score at different periods. Genetic correlations of weights with conformation scores were low but tended to be positive. (AH dl-12)

Selecting for weaning conformation score would be expected to be about .88 as efficient as 550-day score itself in changing genotype for 550-day score. For bulls the correlations of 396-day and 550-day score with genotype for 550-day score were equal. These results indicate that the genetic factors affecting conformation score taken at different ages are to a large extent the same. (AH dl-12)

Correlations of the phenotypes for weight at various ages with the genotype for 550-day weight indicate that some measure of postweaning gain should be included with weaning weight in predicting the genotype for 550-day weight. The pooled estimates suggest that 200 and 396 day weights would be .52 and .81 as efficient, respectively, as 550-day weight itself in selecting for 550-day weight. (AH dl-12)

Another study demonstrated that heritability estimates for growth in yearling cattle were appreciably higher than estimates obtained in early stages of life. Selecting for growth based on the preweaning data would be approximately one-third as effective as direct selection for growth in yearling cattle. (AH dl-14)

Studies continued on physiological characters as possible indicators of potential productivity. In one study, blood components were found to be not consistently correlated with production traits. It is interesting to note that at 500 and 700 pounds body weight, urea nitrogen was significantly negatively correlated with rate of gain but positively correlated with feed per unit of gain. (AH dl-19)

Another study on blood components showed a significant correlation between plasma fat and age of calf. However, correlations between other blood values and initial weight, test gain, final weight, feed efficiency, and final score were nonsignificant. (AH dl-36)

Thyroid secretion rate was estimated in a population of cattle using the I¹³¹ method. There were no differences between sire groups in thyroid secretion rate and the correlation between growth rate and thyroid secretion rate was negligible. The relationship between blood glucose and certain non-sugar reducing substances in the blood with growth rate was not high enough to have predictive value for estimating differences in growth rate.

2. Carcass characteristics. Results continue to show high heritability estimates for many important carcass traits. The greatest problem in effecting genetic improvement is that of estimating carcass characteristics in living animals so that the costly and time-consuming step of progeny

testing can be eliminated. Work on this problem and evaluation techniques on carcasses themselves, will be given in the section on performance testing.

An analysis of steer data involving over 600 head fed during a ten-year period was published and indicated only low and generally insignificant genetic relationships between most carcass characters and production traits. (AH d1-2)

Data from another study indicate that a larger rib-eye area is associated with faster gains and larger size, but is not associated with improved feed conversion, type, increased yield of higher-priced wholesale cuts, or improved eating quality. Correlations between feed conversion and wholesale cut weights and yields were low or negative, as were correlations between performance records and taste-panel scores. (AH dl-8)

While the results are preliminary, there are some indications of a positive genetic correlation between growth rate and carcass fatness. More research is needed in this area but such a relationship seems possible on the basis of appetite phenomena. (AH dl-12)

These studies, as well as others in previous years, indicate that, except when the carcass traits are connected in some way with size, genetic correlations between carcass traits and live production traits are low.

In a comparison of seven breeds and three types - beef type, dairy type, and zebu type - loin steaks from dairy type steers were most tender when evaluated by a Warner-Bratzler shear machine. Most of the advantage in the dairy type was contributed by the Jersey breed, which seemed to be quite tender, both as evaluated by shearing and on a tenderness score evaluated by a panel. However, Jersey steers were least efficient on production, next to lowest on daily gains, and produced the poorest carcass yields. Holstein steers on the same test had the highest daily gains, with the highest feed efficiency, and produced carcasses with high cutting yields. They were average among breeds in eating quality. The Brahmans had a higher percent of separable muscle, but had poor feedlot performance and ranked at the bottom on palatability scores. Santa Gertrudis and Brahman crosses had acceptable gains, feed conversion, and carcass cutability, but were usually ranked next to Brahmans on palatability score. Angus carcasses graded highest of all, but had low cutting yields, largely due to a higher percent of fat. Hereford carcasses had higher cutting yields and were graded slightly higher than Angus on palatability, despite a significantly lower carcass grade and degree of marbling. Both British breeds were above average in palatability. (AH dl-9)

3. Reproductive phenomena. Fertility and its components have generally been shown to be low in heritability but fertility is undoubtedly the single, most important problem of the beef cattle industry. A four-year summary of most herds in the Southern Regional Project, involving several thousand cow years, showed that 77% of cows bred calved and 72% raised calves. Weaning percentage varied among States from 64% to 88%. Industry statistics for the

same area are not available, but indications are that the reproductive rates in experiment station's herds are indicative of industry levels.

In spite of low heritability, breed differences have been observed in the South in reproductive rates with British breeds (Angus, Hereford, and Shorthorn) generally having higher calving percentages than pure Zebu breeds or breeds with Zebu blood (Brahman, Brangus, Santa Gertrudis). The three British breeds studied had a higher conception rate when they were nursing a calf when bred, while the three Zebu breeds had the highest conception rate when they were dry during the breeding season.

4. <u>Sexual maturity</u>. The age at which heifers first come into heat has been studied for different breeds. Of four straight-breds studied - Angus, Hereford, Brangus, and Brahman - the Angus, on the average, came in heat earlier, followed by Hereford, Brangus, and Brahman, respectively. Among backcross heifers, three-quarter Shorthorn heifers reached puberty earlier than three-quarter Angus, three-quarter Hereford, three-quarter Brangus, three-quarter Charolaise, and three-quarter Brahman. No heterosis has been noted for age of puberty in either of these studies. (AH dl-6)

Age of puberty has been determined at the Iberia Station for heifers of Angus, Brangus, Brahman, and Africander-Angus breeding using sterile teaser or fertile bulls painted with a grease paint pigment mixture. Crossbred heifers fed a fattening ration are also checked for age at puberty using sterile males during the drylot fattening period for 168 days. Angus heifers reached puberty at a younger age than heifers of the other breeds studied. The Brangus and Africander-Angus were intermediate while the Brahmans were oldest at time of sexual maturity. None of the Brahmans born in 1960 had shown heat at two years of age while nearly all heifers of other breeds had reached sexual maturity. The first cross (AXB) (BXA) heifers reached puberty at an earlier age due to a faster growth rate than heifers of other breeds. Angussired crossbred heifer calves reached puberty at a consistently younger age than crossbred calves sired by Brahman or Brangus bulls, when fed a fattening ration for 168 days. (AH dl-30)

Age and weight at puberty were determined in 49 straightbred and 54 crossbred heifers at Fort Robinson. These heifers represented progeny for Angus, Hereford, and Shorthorn sires mated to cows of their own breed and to cows of the two other breeds. Heifers were wintered on native grass with one pound of cake. The average age at puberty was 415 days for straightbred heifers and 380 days for crossbred heifers. Average weight at puberty was 525 and 530 pounds for straightbred and crossbred heifers, respectively. The average age and weight were 444 days and 574 pounds; 405 days and 513 pounds; 395 days and 489 pounds for Hereford, Angus and Shorthorn heifers, respectively. (AH dl-37)

At Miles City, studies on age and weight at sexual maturity in both heifers and bulls are being conducted. Bulls and heifers represent the Hereford, Angus, and Charolaise breeds. In addition, crossbred and reciprocal cross-

bred animals resulting from the interbreed mating of these three breeds and crossbreds from matings of Hereford, Angus, and Charolaise bulls on Brown Swiss cows are also being studied. The study involves 83 heifers, of which 25 are straightbred and 58 are crossbreds. In the heifers, 92 percent of the straightbreds and 95 percent of the crossbreds have exhibited estrus. Average age at first estrus was 355 days for both straight and crossbreds and average weight at first estrus was 625 and 629 pounds for straight and crossbreds, respectively. Age and weight of heifers classified by breed of sire was 365 days and 630 pounds, 346 days and 595 pounds and 346 days and 660 pounds for Hereford, Angus, and Charolaise sires, respectively.

Age at puberty has been determined in 21 straightbred and crossbred bulls. All bulls are subjected to electroejaculation at 28-day intervals and the ejaculate examined microscopically for the presence, motility, and concentration of sperm. Fourteen days after electroejaculation and again at 28-day intervals all bulls are exposed to an estrogen treated heifer and breeding ability determined and a libido score given.

To date all bulls have reached puberty except one straightbred Charolaise. Crossbred bulls have reached electroejaculation classification criteria earlier than straightbreds as shown by the following averages. Age (days) for purebreds vs. crossbreds at: appearance of sperm, 243 vs. 228; appearance of motile sperm, 285 vs. 248; appearance of sperm numbers sufficient for detailed classification, 276 vs. 260; and first definite ejaculation; 313 vs. 288, straightbreds vs. crossbreds, respectively. In breeding ability data, average puberty age and libido scores were 375 days and 3.0 vs. 355 days and 3.2 for straightbreds vs. crossbreds, respectively. (AH dl-33)

Variation of milk production between and within breeds is being studied. There is additional evidence that a significant relationship exists between milk production of the dam and calf gains. A study at the Texas station has shown no noticeable decline in milk production through the lactation period in beef cows, as has been reported in dairy cows. This station also reports that milk yields within breed vary from slightly less than two pounds to more than 18 pounds of milk during mid-lactation. These data come from some 400 cows of Angus, Brahman, Hereford, Shorthorn, Santa Gertrudis, half-Brahman, half-Hereford, and half-Charolaise breeding. In a comparison of breeds at the Jeanerette station, Brangus cows yielded more milk - followed by Angus, Africander-Angus, and Brahman, respectively. (AH dl-6, dl-22, dl-29)

5. Reproductive rates in breeding herds. Results from the 1962 breeding season at fort Robinson show that cows being bred for straightbred calves had slightly superior reproductive performance as compared to cows being bred for crossbred calves. These results are not consistent with the results obtained during the past three years. The proportion diagnosed pregnant was 95% for cows bred for straightbred calves versus 93% for cows bred for crossbred calves. Little difference was noted in the number of services required per conception or the percent settling at first service.

A comparison between reproductive performance of crossbred and straightbred cows, both bred for crossbred calves, has also been obtained this past year. The proportion of cows pregnant was 97% for crossbred cows versus 90% for straightbred cows. Fewer services per conception (2.00 vs. 1.76) were required by the crossbred cows and more settled on first service (60% vs. 57%). The embryonic mortality was much higher in straightbred cows than in the crossbred cows (15% vs. 3%). The proportion of crossbred cows giving birth to a calf was 93% vs. 77% for the straightbred cows. (AH dl-37)

The pregnancy rate of the breeding herd for 1962 at Jeanerette was about average with pregnancy rates of previous years but was much lower than for 1961. The proportion of the cows pregnant was 87, 63, 65, 72, and 100 percent for the Angus, Brangus, Brahman, Africander-Angus, and first cross (AXB) (BXA) cows, respectively. Two-year-old heifers had a higher fertility rate (79% pregnant) than three-year-old (59% pregnant) or older cows (73% pregnant). Only 27 and 33 percent of the three-year-old Brangus and Branman heifers bred, were diagnosed pregnant compared to percentages of 67 and 86 for the Africander-Angus and Angus, respectively.

A major cause of low fertility was lack of estrus during the 75-day breeding season. Cows not showing heat during the breeding season accounted for 37 percent of the open Brangus, 67 percent of the open Brahman, and 86 percent of the open Africander-Angus. Forty-seven percent of the open cows which showed heat had only one or two heat periods. The interval from calving to first heat was 85, 130, 200, 122, and 71 days, respectively, for Angus, Brangus, Brahman, Africander-Angus, and F1 crossbred three-year-old lactating heifers. Older cows had shorter intervals from calving to first heat than three and four-year-old cows. Intervals from calving to first breeding and to conception were longer for cows with Zebu breeding than Angus cows.

The proportion of cows which conceived was 60%, 51%, 21%, and 21% for first through fourth services, respectively. Of the cows which settled during the breeding season, 96.3% did so the first 64 days. There would appear to be little merit in breeding longer than this time. The number of heat periods previous to breeding appeared to influence conception rate. The proportion pregnant was 52, 60, 70, and 50% for cows bred at their first, second, third, or fourth heat period, respectively. (AH dl-30)

6. Genetic-environmental interactions. The interregional project involving selection of originally similar base stocks in two different environments (Miles City, Mont., and Brooksville, Fla.) has progressed with the final cattle transfers for setting up the base herds scheduled for the fall of 1963. To date, cattle from each location have apparently adapted reasonably well to the other within a few months to a year. (AH dl-41)

A study in which possible differential response of sire progenies was studied when heifers were fed limited grain rations on pasture and bulls full-fed in drylot showed no evidence of significant genetic-environmental interaction. (AH dl-8)

7. Genetic defects. Work continued on a reduced scale during the year on possible methods of detecting animals heterozygous for snorter-type dwarfism but with no positive results. (AH dl-9, dl-10, dl-12, dl-22, dl-31)

Work is being continued on mucopolysaccharidosis in dwarf cattle. It has been noted that matings of the Snorter dwarf bulls and cows of mixed breeding (Brahman-native) produce a ratio of dwarf to normal calves of less than 1:1. These results suggest that genes carried by animals of mixed breeding modify expression of the Snorter dwarf gene. (AH dl-34)

Greater accuracy is being achieved in recognizing specific achondroplastic types so that appropriate classifications can be made, thereby improving accuracy. These methods are a considerable improvement over visual classification. Possible reasons why certain so-called pedigree clean lines break down are being studied. There is a possible connection between dwarfism and at least certain types of hydrocephalus. A new small type of achondroplastic has been discovered which shows slender long bones, extreme curvature of the spine, and a slightly later fusion of the sphenooccipital synchondrosis. (AH dl-39)

Defects including hydrocephalus and a bulldog-type monster have occurred in inbred lines with no previous history of the production of such defects. (AH dl-2, dl-4).

B. Performance Testing

Attention to improving methods for evaluating performance in beef cattle is continuous in most projects. The most significant overall recent trends are increased attention to (1) carcass evaluation, including methods of estimating carcass characteristics from live animals, and (2) evaluation of fertility and the components or factors upon which it depends. Routine evaluations of these traits will make more comprehensive future genetic analyses possible.

1. Carcass traits. An effort has been made during the past year to collect considerable data on the use of ultrasonics as a tool in live animal carcass evaluation. Indications are that this may be a promising tool for the measurement of fat thickness in the live animal. Evidence in the past has shown that fat thickness and carcass weight make a fairly good predictive measurement of total muscle in the carcass. If a good estimate of fat thickness in the live animal could be obtained, faster progress could be made in selection for muscling. Correlations of estimated fat thickness by ultrasonic techniques with actual fat thickness taken on the carcass have ranged from a low of 0.2 to a high of 0.9. Correlations between estimated rib-eye area and actual rib-eye area, as traced on the carcass, have, in general, ranged from 0.4 to 0.7. One station has indicated that repeatability of measurement between operators seems to be quite high, while evidence from another station indicates this repeatability to be of a much lower magnitude. (AH dl-9, dl-12)

A three-dimensional photographic system known as "photogrammetry" is being studied to see if live animal photographs can be used to predict weights and proportions of wholesale cuts. In weight-variable populations the correlation between actual and predicted weights of wholesale cuts has been high but the correlation between live weight and weight of cuts has also been high leading to the conclusion that the photographic technique is an excellent indicator of size. Variability in percent of different wholesale cuts from animal to animal is relatively small and it is uncertain how well the technique can pick up proportional differences in weight-constant groups. (AH dl-2, dl-10)

In one study a correlation of .85 between predicted and actual backfat thickness was observed from use of a probe in live animals. (AH dl-20)

It is apparent that problems of technique must be solved if this procedure is to be effectively utilized by the industry.

Further study of correlations between subjective estimates of fat thickness and rib-eye area in slaughter cattle with measures of these traits in the carcass have again averaged approximately .4 to .5. The correlations between live estimates of yield of trimmed retail cuts from the round, loin, rib and chuck and actual measures by carcass cut-out have averaged approximately .5. This was among slaughter cattle of rather uniform weight and condition. While some results indicate that differences in rib-eye area are a rather poor measure of differences in muscling in the carcass, results also indicate variations in rib-eye area of considerably greater magnitude than variations in loin length. (AH dl-12)

In one study increases in length of body, depth of fat, depth of round, and carcass weight increases were linear when breed, sex, and years were held constant. Area of rib-eye tended to level off at higher weights. Therefore, carcass weight in itself gave an accurate prediction of length of body and depth of round when breed, sex, and years were held constant, but the prediction was not as high for area of rib-eye. In a study of growth of body parts, it appears that the length of some of the muscles starts leveling off at 360 days. Weight of muscles continues to increase in direct proportion to carcass weight. (AH dl-25)

2. Growth rate. A major problem of beef cattle breeding is the adjustment of preweaning records of calves for sex of calf and age of dam so that valid comparisons can be made between calves from dams of different ages and between sire progenies and different cows. If universally applicable adjustment factors could be developed, progress would be increased. However, data from several stations have shown that environmental effects have different influences on different breeds and at different locations. Similarly, age of dam effects have been found to be different in different years and, in some cases, differential age of dam effects on sex have been observed. This indicates that it is important not to use the same correlation factors regardless of breed, location, age, etc. (AH dl-6, dl-16, dl-20)

As an example of the foregoing, an analysis was made of records accumulated at one station over a period of more than 25 years and the records divided into "high" and "low" years on the basis of average weaning weights. Weights of calves from three-year-old dams were 37 pounds below those from mature dams in the low years but only 11 pounds in the high years. In this herd an adjustment system which will take year average into account appears feasible but its applicability to other areas is not known. (AH dl-6)

One study indicates that, as would be expected, cows with heavier mature weights tend to wean heavier calves. Thus, it would appear that selection for calf weights should be in relation to weight of dam. (AH dl-2).

A limited amount of data from another study indicates a curvilinear regression of weight of calf upon weight of dam. The heaviest calves were produced by cows which weighed from 1100 to 1200 pounds. Data from the same study revealed that maximum cow weight was reached at 10 to 11 years. Data on several thousand head of Santa Gertrudis cattle belonging to a private breeder revealed that the Santa Gertrudis Breeders' International classification of "S" and "S=" was found to have highly significant association with both weaning weight and weaning type. These data indicate that those factors influencing the breed classification at long yearling age also exert a significant influence on weaning performance of the offspring. (AH dl-22)

C. Selection and Systems of Breeding

1. Crossbreeding. Measures of heterosis effects involving reciprocal crosses among the Angus, Hereford and Shorthorn breeds continue to show that the crossbreds grow somewhat faster with slightly less feed required per 100 pounds of gain than the straightbreds by the same sires. The crossbreds had more fat trim (in percent) than the straightbreds at the same age; however, there was no difference in carcass grade. The crossbreds had approximately \$6 adv ntage in net carcass value based on pounds of retail product produced (adjusted for differences in quality grade) and feed costs from weaning to slaughter. The crossbreds reached puberty at younger ages that the straightbreds. (AH dl-12)

In the southern region, analysis of data from crossbreeding experiments continues to indicate that crossbred offspring show some heterosis over the average of the parent breeds. An analysis of 180-day calf weights at one station indicated there was a substantial advantage of the crossbreds over the average of the purebreds, 15.%. At the same station, backcross calves by crossbred dams were 18.% heavier than the average of the purebreds. The data from this station reveal an apparent interaction between breed or cross and age of dam. Hereford, Brahman, and first-cross dams of these two breeds exhibit markedly different response curves due to age. Heterosis was also exhibited in the feedlot gains when calves were put on full feed. This heterotic effect has been shown to be as much as 11% in some cases. (AH d1-22)

Data from other stations indicate that crossbred steers were as much as 41 pounds heavier at weaning time than the parent breeds. (AH dl-29)

At several locations, work with British breed crosses has progressed to the point that preliminary information is being obtained beyond first crosses, i.e., on three-way and backcrosses. Generally speaking, the crossbred dams appear to be productive and three-breed crosses exhibit somewhat greater growth than backcrosses. (AH dl-12, dl-7)

Reproductive efficiency in first crosses between British breeds has varied between studies but, on the average, fertility has been higher in cows bred for crossbred calves than in those bred for straightbred calves. In most experiments death losses of crossbred calves have been less than of purebreds leading to an average greater net calf crop for crossbreeding. (AH dl-1, dl-3, dl-7, dl-12, dl-29)

In British-Brahman crossing tests, the crossbred cows have had marked advantages in fertility and calf survival as compared to averages of purebred parental types which, when considered in connection with the greater growth rate of their calves, resulted in about 25% more pounds of calf weaned per cow bred. The crossbred cows are also giving indications of having a longer productive life. (AH dl-22)

Crosses of the Charolaise breed with British breeds have usually resulted in considerably increased growth rates as compared to British types but in one experiment no advantages were found in Charbray - Hereford crosses. (AH dl-1, dl-40)

2. Inbreeding and linecrossing. Development of inbred lines continued at several locations with general observations being that inbreeding is usually accompanied by some reduction in fertility, livability and growth rate. In one study, estimates of line x mating system interaction indicate a greater heterosis in females. This increase in heterosis is not associated with degree of inbreeding depression. A curvilinear response to inbreeding has been demonstrated in these data. This response differed between the two sexes. Regression studies showed that inbreeding of dam has a greater effect on weaning weight than inbreeding of calf. Postweaning traits were not significantly affected by inbreeding with the exception that inbreeding of calf significantly affected yearling traits. (AH dl-2, dl-4, dl-16)

In linecrossing experiments within the Hereford breed, crossline bulls were 19 pounds heavier at weaning time than straight-line contemporary bulls. Crossline heifers were 29 pounds heavier than straight-line heifers. (AH dl-2)

Additional data show that, in general, station inbred lines developed with concurrent selection for traits of productive importance, have shown advantages in growth when top-crossed in commercial herds. (AH dl-13, dl-17)

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BEEF CATTLE - PHYSIOLOGY Animal Husbandry Research Division, ARS

Problem. In the average lifetime of a beef cow, under optimum conditions, she will wean only 5 or 6 calves. Under suboptimum conditions, she may wean as few as 3 or 4 calves. In most cases the calving season will last at least 3 or 4 months and calving for 12 months or on a year-round basis is not uncommon. Cows or heifers not showing heat during the early part of the breeding season and a low conception rate at first service are two of the major causes of low reproductive performance. Methods must be found to improve reproductive efficiency so that the number of calves weaned per cow will be higher and cows will calve over a relatively short period of time, thus yielding more pounds of calf. Also, more information concerning physiological processes involved in feed conversion could lead to more efficient animals. Furthermore, data on effect of climatic conditions on physiological response of cattle are needed. These data could be utilized to provide information so management practices could be altered to increase production.

USDA PROGRAM

The program at the present time is mainly concerned with methods of improving, controlling or altering reproductive performance by hormonal, nutritional or other methods. It is carried on by physiologists and animal husbandmen at Beltsville, Maryland, and at the Department's Fort Robinson, Nebraska; Miles City, Montana; Jeanerette, Louisiana, and Fort Reno, Oklahoma, stations in cooperation with the respective State Experiment Stations. Studies on the causes of reproductive failures are conducted with the herds at all these locations. Investigations on the relationship between reproductive performance and protein and energy intake levels are in progress at Beltsville, Fort Robinson, Jeanerette and Miles City. Also at Beltsville, studies are in progress on the reproductive performance of cattle exposed to high temperatures and humidity. Studies are underway at Miles City and Fort Robinson to determine the relationship between anatomy of the pelvis and calving difficulties. Other studies at Fort Robinson include work on control of the estrous cycle and the effect of low doses of estrogenic hormones on ovarian activity.

The <u>Federal</u> scientific effort devoted to research in this area totals 3.5 professional man-years of which 3.1 are devoted to <u>physiology of reproduction</u> and .4 to program leadership.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Physiology of Reproduction

1. Nutrition and reproduction. Effect of protein and energy on reproduction is being studied at the Agricultural Research Center, Beltsville Maryland. Fifty-eight grade Angus heifers, weighing an average of 420 pounds were allotted to six treatments ranging from a full-feed (approximately 18 pounds) to 4.6 pounds of feed daily. After 59 weeks on experiment the heifers vary in body weight from an average of 960 pounds for the heifers fed ad lib. to 470 pounds for the heifers fed the lowest level. All animals have shown estrus with the exception of three heifers in the two groups receiving the lowest level of nutrition; also six heifers in these groups ceased cycling after showing estrus. These heifers were raised to a higher level of nutrition to bring them back into heat. One hundred percent of the heifers have been bred in the four higher level groups while 90 and 28 percent of the heifers on the two lower levels of nutrition have been bred. The conception rate for all heifers regardless of nutritional level has been normal. serum protein, which is determined every six months, appears to decrease with time in all groups except in the heifers in the ad lib. group. (AH d2-22)

The effect of four levels of winter feeding and two levels of summer grazing on reproductive performance of Hereford heifers bred to calve at two years of age was studied at Fort Reno Experiment Station in El Reno, Oklahoma. All heifers on high or moderate levels of winter feeding showed heat during breeding season. Seven of the 15 heifers on low levels of winter feeding and restricted summer grazing did not show heat during breeding season. Heifers on higher levels of winter feeding tended to reach puberty sooner. If first heat was exhibited during winter months, regular estrous cycles tended to be established in most of the heifers on high and moderate levels of winter feed but in very few of the heifers on low levels of feed. Most of the heifers (93 or 100%) on high or moderate levels of winter feed or on low winter feed and high levels of early spring feed became pregnant during the breeding season. In heifers on low levels of winter feed and continuous summer grazing, 73% became pregnant while 53% of those on low levels of winter feed and restricted summer grazing were diagnosed pregnant. Conception of heifers conceiving on low levels of winter feed occurred later in the breeding season These results show that low levels of winter feed delay the onset of sexual maturity. This delay leads to unsatisfactory reproductive performance especially when it is associated with low levels of summer grazing. (AH d2-12)

Work at the Iberia Livestock Station at Jeanerette, Louisiana, indicates low reproductive performance in young lactating cows is the result of insufficient nutrients being supplied by pasture during winter and early spring months. The percent of lactating two-year-old heifers becoming pregnant in a 100-day breeding period was Group 1 (pasture only) 54%; Group 2 (drylot weight gains equivalent to pasture only) 46%; Group 3 (fed NRC recommenda-

tions in drylot) 85%; Group 4 (pasture plus supplement to give weight gains equivalent to weight gains of Group 3) 82%.

The major cause of reproductive failure was a delay in the onset of heat following calving. Fifteen percent of the cows in Group 1 and 8% of the cows in Group 2 did not show heat during the breeding period. In addition, the percentage of cows showing heat by the 43rd day of the breeding season was 15%, 8%, 62%, and 64% for Group 1 through 4, respectively. The interval from calving to first heat for cows showing heat prior to the end of the breeding season was 122, 141, 113, and 100 days, respectively, for Group 1 through 4. The proportion of the lactating heifers conceiving at first service was 45% and 36% for Groups 1 and 2 compared to 62% and 73% of cows settled at the first service; Groups 3 and 4. Feed provided to animals in Group 2 (drylot) to equal gains of the pasture only (Group 1) varied from a TDN of 4.5 in January and February to 16.8 pounds in April and May. Heifers, therefore, appeared to obtain insufficient energy in the winter and early spring months to support optimum reproduction. (AH d2-34)

An experiment is being conducted at Fort Robinson, Nebraska, to determine the effect of different levels of energy before and after calving on reproductive performance of heifers calving at 2 years of age. Two hundred and forty Angus and Hereford heifers are being used. Before calving a high level (8.3 lbs. of TDN) and a low level (4.1 lbs. of TDN) of energy are being fed. The high group before calving is divided into three groups after calving; full-fed (approximately 19.0 lbs. of TDN) and a high group (13.0 lbs. of TDN).

Cows are placed on experimental rations 140 days before calving. Marked differences in body weights and body condition have been noted. Cows on low levels of feed prior to calving lost approximately 40 lbs. and 2 condition scores while cows on high levels of feed gained 120 lbs. and condition remained constant. Data on reproductive performance are not yet available. (AH dl-37)

A study of the effects of energy level on growth and reproduction of range heifers is being conducted at the Range Experiment Station at Miles City, Montana. The study involves 48 Hereford heifers grazed on range throughout the trial with supplemental feeding divided into two phases. During the 140-day wintering phase the heifers were individually fed, every-other day, either two pounds of a 40% protein pellet or 4 pounds of a 20% protein pellet. Rations were formulated to provide equivalent amounts of protein, vitamin A and phosphorus but differing in energy content. The second feeding phase consists of a 56-day prebreeding feed period in which both wintering ration groups were divided into groups receiving either range only or four pounds of the 20 percent protein pellet fed individually every other day, thus giving four final nutritional regimes, LL, LH, HL, and HH.

Results to date have not indicated marked differences in body growth measurements or weight gains. Reproductive tract growth has been similar among ration groups but there is some evidence that estrual activity is greater in

heifers receiving higher levels of energy during the prebreeding period. Number of heifers in heat are 3, 4, 1, and 5 for LL, LH, HL, and HH, respectively.

All heifers will be exposed to a fertile bull for a 60-day breeding season and the number of heifers bred and conception rates will be studied. (AH dl-33)

2. Control of estrus. Attempts have been made to synchronize estrous cycles at Fort Robinson, Nebraska. Eighty cycling heifers received 18 daily injections of various combinations of progesterones and estradiol (20 mg. progesterone with 80 mcg. and 160 mcg. of estradiol and 40 mg. progesterone with 80 mcg and 160 mcg. estradiol). The percent synchronized over a four-day period was 95 and 100% for the two groups. The conception rate at the synchronized estrus ranged from 20% to 32% compared to 58% for a control group.

Forty cycling heifers were individually fed oral progesterone (droxone from E. R. Squibb and Co.) twice each day. The four dosage levels were 4 grams, 2 grams, 1 gram, and 1/2 gram. The proportions showing estrus in a four-day period following termination of treatment were 100, 70, 90, and 80 for the 1/2 gram, 1 gram, 2 grams, and 4 grams, respectively. The proportion pregnant varied from 10 to 22%.

Ninety heifers were given a single injection of a long acting progesterone (droxone) or a combination of a long acting progesterone with a long acting estrogen (estradiol enanthate). The levels used were: 62.5 mg. of droxone with 0; 0.125 mcg. or 0.25 mcg. estradiol enanthate; 125 mg. of droxone with 0; .25 mcg. or 0.5 mcg. estradiol enanthate; 250 mg. droxone with 0; 0.5 mcg. or 1.0 mcg. estradiol enanthate. Poor synchronization was obtained with 40 to 50% of the heifers showing estrus in a four-day period. Fertility ranged from 0 to 33%.

Results from these trials indicate that good synchronization can be obtained by feeding or daily injections. However, fertility at the synchronized heat is poor. (AH dl-37)

A study to determine causes of maintenance and regression of corpus luteum has been conducted at Fort Robinson. Ninety-nine yearling Hereford heifers were assigned to a cycling, pregnant or hysterectomized group to study the effects of various estrogens on ovarian activity. Single intramuscular injections of either 50 mg. estradiol - 17 beta; 50 mg. estradiol - 17 alpha; 50 mg. estrone; or 5 mg. estradiol valerate were administered on day 5 in the cycling and hysterectomized heifers and on day 35 in the pregnant heifers. Heifers were laparotomized or hysterectomized and corpora lutea marked with India ink at the time of hormone treatment. Ovariectomies were performed 7 days after the hormone treatment. Estrogen treatment significantly reduced corpus luteum weight, progesterone concentration, progesterone content and ovarian follicular fluid weight in all three reproductive groups. Average

values for the cycling, pregnant and hysterectomized control groups were: 3.06, 3.03 and 3.32 gm. for corpus luteum weight; 17.9, 15.9 and 28.6 mcg./gm. for progesterone concentration; 55.1, 47.4 and 93.7 mcg. for progesterone content; and 4.32, 3.36 and 2.94 for follicular fluid weight, respectively. Average values for the treated groups ranged from 1.88 to 2.53 gm. for corpus luteum weight; 7.5 to 18.4 mcg./gm. for progesterone concentration; 15.1 to 45.4 mcg. for progesterone content; and 2.17 to 2.77 for follicular fluid weight. Estradiol - 17 beta was the most effective estrogen while estradiol - 17 alpha was the least effective. Heifers of different reproductive states (cycling, pregnant and hysterectomized) responded similarly to the different forms of estrogen administered for all of the indices of ovarian function studied (reproductive state x hormone treatment interactions; small and non-significant). Thus, no evidence was obtained to support the hypothesis that the uterus affects ovarian function by metabolic utilization or transformation of estrogens. (AH dl-37)

- 3. Temperature effects on reproduction. At Beltsville, in December, 1962, six Hereford heifers were placed in a psychrometric chamber at 90 F. and 60% relative humidity. All heifers stopped having estrual cycles after being subjected to the heat stress. After seven weeks, two heifers had to be removed from the chamber because of nerve damage to the rear legs. Body temperatures and water consumption rose to high peaks by the fifth week and at this time declined along with the shedding of the hair coat. These reactions were similar to the other trials indicating adaptation is important in their response to heat stress and the resulting effect on the estrous cycle. Three of the four remaining heifers reestablished their estrous cycles after 16 weeks exposure to heat stress and have been bred. (AH dl-30)
- 4. Losses at or near calving. In efforts to ascertain causes of still-births the area of the dam's pelvic opening is being determined in all first calf heifers at Miles City. In addition to internal pelvic measurements, external measurements in terms of hip width and rump length are also being determined. These measurements will be correlated with each other and with severity of calving difficulty. To date, age differences within breeds have been noted as shown by comparing Hereford two and three-year-olds, respectively, as to: area of pelvic opening, 228.3 vs. 289.9 sq. cm.; hip width, 17.2 vs. 20.4 inches; and rump length, 17.3 vs. 19.6 inches. Breed differences within age groups are also apparent as shown by comparing three-year-old Herefords with three-year-old Charolaise, respectively: area of pelvic opening, 289.9 vs. 328.0 sq. cm.; hip width, 20.4 vs. 21.6 inches; rump length 19.6 vs. 20.7 inches.

Calves from first calf heifers are being measured within 24 hours following birth to determine width of head, shoulders and hips and depth of chest. These measurements will be used to study breed differences in calf size and to estimate what might be considered a "minimum" pelvic opening. (AH dl-33)

5. Reproductive phenomena. In a study of reproductive rates in the southern region over a four-year period, several things of an "environmental" nature were shown to be related to fertility. They would have to be taken into account in comparing fertility of individuals or sire progeny groups.

On the average, cows that were nursing a calf when bred weaned approximately four percent more calves during the subsequent season than did cows which were dry when bred. Four-year-old cows, when bred, weaned four and one-half percent more calves than the average. Yearlings were approximately one percent below the average, two-year-olds were two percent below, and three-year-olds were approximately one percent below. In this four year study, pasture mating of cows resulted in approximately 36 percent more calves than did artificial insemination. There was little difference in percent of conception between hand mating and pasture mating. It is interesting to note that approximately eight percent of the cows in this study were removed each year for reproductive causes.

In one study on cows that did not conceive during the regular breeding season, it was noted that during a subsequent 27-day breeding period, cows nursing calves had a lower pregnancy percent (43%) than cows not nursing calves (62%). It was also noted that supplemental feeding further increased the conception rate of these cows. A study was conducted at the same station on the relationship between the growth rate of heifers up to two years of age and their subsequent calving percent at three years of age. In general, lightweight calves at weaning had lower subsequent fertility rates than heavier calves. (AH dl-6, dl-30)

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Physiology of Reproduction

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- Kaltenbach, C. C. 1963. Alteration of ovarian activity in cycling, pregnant and hysterectomized heifers with exogenous estrogens. M.S. Thesis, University of Nebraska Library. (AH dl-37)
- Reynolds, W. L. 1963. The age and weight at puberty of Angus, Brahman and Zebu cross heifers. J. Anim. Sci. 22: 243 (Abstr.) (AH dl-30 (rev.))
- Turman, E. J., Pope, L. S., Watkins, B. J., Pinney, D. O., McNutt, D. D., and Stephens, D. F. 1963. The reproductive performance of Hereford heifers on different levels of winter feeding and summer grazing. Okla. Agric. Exp. Sta. Misc. Pub. 70. (AH d2-12)

- Wiltbank, J. N., Rowden, W. W., Ingalls, J. E., and Zimmerman, D. R. 1962. Influence of post-partum energy intake on reproductive performance of Hereford cows restricted in energy intake prior to calving. Am. Soc. of An. Prod. West. Sect. Proc., 13: X. (AH dl-37)
- Wiltbank, J. N. and Zimmerman, D. R. 1962. Estrus synchronization and fertility in Hereford heifers subsequent to administration of progesterone and estradiol. Am. Soc. of An. Prod. West. Sect. Proc. 13: XVII. (AH dl-37)

BEEF CATTLE - NUTRITION AND MANAGEMENT Animal Husbandry Research Division, ARS

Problem. Producers of beef cattle need improved feeding methods which will result in optimum pasture gains, reduced feed consumption per pound of beef produced, optimum reproductive rates and desired carcass qualities. To meet these needs basic nutritional information is required such as: When should beef animals be fed for maximum gains and when for more limited gains? What nutrient combinations produce rapid growth of muscle with a minimum of fat deposition? How may breeding animals be economically raised that will be capable of a high level of reproductive performance over a long lifetime? What are the nutritive contributions made by range and pasture and what supplementation is required when they are used? Research is also needed on the relation between animal production and types of shelters and equipment, feeding systems, and methods of increasing labor efficiency.

USDA PROGRAM

This is a continuing program carried on by nutritionists, biochemists and animal husbandmen on basic and applied problems related to feeding and management of cattle for beef. The work is in progress at Beltsville, Maryland; in cooperation with State Experiment Stations at federally owned stations in Miles City, Mont.; Fort Robinson, Nebr.; Fort Reno, Okla.; Jeanerette, La.; Brooksville, Fla.; and Front Royal, Va.; and in cooperation with State Experiment Stations at Raleigh, N. C.; Tifton, Ga.; College Station, Tex.; and Newell, S. Dak.

The Federal scientific effort devoted to research in this area totals 10.3 professional man years. Of this number 2.7 are devoted to digestion and metabolism; .6 to concentrates; 2.7 to forage preservation and utilization; 1.4 to nutrient requirements; 1.0 to range and pasture management; .7 to management practices; and 1.2 to program leadership.

There is one grant involving Public Law 480 funds with the Agricultural College in Poznan, Poland. The project is on content of trace minerals in forage crops in relation to the stage of development of the plants and the method of gathering and storing. It is supported for five years (1963 to 1968) by \$47,311.66 equivalent in Polish zlotys.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Digestion and Metabolism

1. <u>Digestion techniques</u>. Data collected since 1957 on application of the chromic oxide technique for estimating digestibility of complete, mixed, and pelleted rations have been summarized. Small but significant differences between total collection and indicator techniques for esti-

mating digestibilities were observed. Variances calculated for daily digestion coefficients were greater for values determined by the total collection procedure than by the Cr₂O₃ techniques. Incorporating Cr₂O₃ in a pelleted, complete ration and taking fecal grab samples at 9 a.m. and 3 p.m. for 5 days are considered to be a satisfactory technique for estimating dry matter digestibilities with steers.

At North Carolina, work has continued on the relation of chemical composition to digestibility. The research indicates if one could determine "nutritive entities" of feed, the chemical composition (%) of the entity would be linearly related to the digestible amount of that entity, the slope of the line would be the digestion coefficient and the intercept would be the metabolic amount of that entity. Five different feeds have been used in digestion trials and the results show that crude protein except for lespedeza fits the theory quite well; so do ether extract, ash, water-soluble carbohydrates, and 4% H₂SO₄-hydrolysable carbohydrates. The main problem still remains in the complex carbohydrate fraction.

Work has continued on the determination of the properties of the enzyme inhibitor which is present in sericea lespedeza. Its molecular weight is apparently between 10,000 and 15,000. In vitro it is inhibitory to cellulases, pectinases, pectin-esterase, amylases, and proteinase.

Identical twin heifers are being used to determine if the feeding of a purified diet, which contains urea as the only nitrogen source, will alter growth and reproductive performance. One heifer of each set is being fed a natural ration which contains 75% concentrates while the remaining heifer is being fed a purified ration of the following composition in percentage: corn starch, 28.6; corn sugar, 28.6; cellulose, 30.0; urea, 4.2; soybean oil, 2.0; mineral mix, 6.5; choline chloride, 0.1; and vitamins A and D. These rations are being fed on an isocaloric and isonitrogenous basis. The heifers were started on the rations weighing about 275 lb, each and after 196 days on experiment the heifers on the purified diet gained 0.80 lb. daily while those on the natural ration gained 1.20 lb. daily. Skeletal measurements after 180 days on ration indicated that the heifers on the purified rations were increasing in skeletal size slightly faster than the other heifers. After the heifers obtain a weight of approximately 600 lb. they will be bred and further data will be obtained during gestation and after calving while still consuming the same rations. (AH d2-8, d2-14)

2. Pasture bloat in beef cattle. Research on factors contributing to pasture bloat at Beltsville and at Mississippi State College has been continued. Ethanol precipitated slime fractions have been isolated from centrifuged ruminal fluid of steers bloating on clover pastures. The amount of slime increased during grazing when the animals bloated. Analysis showed the fractions contained 61 to 64% protein, 8 to 14% carbohydrate and 7 to 10% ribonucleic acid. The slime fraction contained 17 amino acids, the sugars d-ribose, arabinose, and glucose and the purines and pyrimidines

adenine, guanine, cytosine, and uracil. Clover cytoplasmic protein has been implicated as a major source of stable foam in pasture bloat. In feedlot bloat, increases in dry weight of the ethanol insoluble material have been related to the onset and severity of bloat. The ethanol-insoluble fraction in clover bloaters showed approximately the same range of increases before and after bloat as did animals bloating on grain. The slime fraction of the legume bloaters and of the feedlot bloat animals was similar in having a protein, carbohydrate and nucleic acid composition but differs in the kind of nucleic acid component. In the grain bloater the nucleic acid is of the DNA type while in the legume bloaters the nucleic acid in the slime fraction is of the RNA type. The occurrence of protein, carbohydrate, and nucleic acid in the slime fractions of both the grain and legume bloaters, and the increase of the slime with the onset of symptoms suggest these compounds may contribute to the development of bloat. (AH d2-13)

3. <u>Urinary calculi</u>. Basic and applied studies into the cause and prevention of urinary calculi were conducted with both steers and wethers at College Station and Big Spring, Texas, and with steers at Fort Reno, Oklahoma.

The steer studies at College Station were part of a feeding trial comparing rations of ground moist sorghum grain or ground moist sorghum heads with and without Coastal Bermuda haylage. While there were no clinical cases of calculi, 43 of the 50 steers in this test did have stones in the bladder at the time of slaughter.

In all treatments, the number of animals with stones was much too high. Insofar as quantity is concerned, there was no significant difference among the treatments using haylage, but there were significantly greater numbers of stones in steers not fed haylage than in steers fed haylage.

The Fort Reno work was part of a study concerned with the utilization of sorghum grain as related to the method of feeding and the data were developed from sire groups and cross treatments. Five of the 74 steers used in this test developed clinical cases of urolithiasis and 26 more had stones in the bladder at the time of slaughter. Three of the five clinical cases were in one sire group while the fourth case was sired by a half brother. Ten of the 16 steers by these two bulls developed stones and the offspring of these two bulls accounted for 80% of the clinical cases and 30% of all the steers affected. While the numbers are small, it does indicate the possibility of certain blood lines being susceptible to calculi formation. The combined steer and wether experiments at Big Spring made up the second year of a three-year test attempting to resolve or confirm the apparent difference in response of the steer and the wether to factors involved in calculi formation. There were no clinical cases of calculi in the steers. However, all of the steers fed the basal ration and the basal ration plus 1% disodium phosphate had stones in the bladder. The addition of 1% Carbotex (a commercial ground limestone product) resulted in a 37% decrease in the number of steers having stones in the bladder while the addition of 1.5 oz. per head daily of ammonium chloride reduced the incidence by 63%.

In contrast, wethers receiving a ration prepared from the same ingredients used in the steer ration had only one animal in the basal ration treatment group develop stones. Nine clinical cases of calculi developed during the test. Eight of the lambs receiving disodium phosphate and one from the Carbotex group developed stones. None of the lambs receiving ammonium chloride had stones in the bladder at the time of slaughter. While definite conclusions are being withheld pending the completion of all three years of the study, it would appear that different dietary mineral ratios or mechanisms may be involved in calculi formation in the steer than in the wether, but ammonium chloride seems to offer an effective means of the control of urolithiasis for both the steer and the wether. The addition of disodium phosphate to the wether ration definitely increased the calculogenecity as shown by a 5% incidence for the control or basal ration and a 65% incidence when the disodium phosphate was added.

The studies at College Station with wether lambs were designed to test the effect of pelleting, the size of the pellet, and the addition of sodium or potassium chloride to the ration. The results of pelleting were slightly different than observed in previous work. Calculogenecity was increased when the ration was pelleted which corresponded to previous observations. However, the greatest increase was found in the 3/8 in. pellet instead of the 1/4 in. pellet. Also the lambs fed the 1/4 in. pellet gained essentially the same as the lambs fed the 3/8 in. pellet and at about the same feed efficiency. Combining the two year's work insofar as calculogenecity is concerned, 38% of the lambs fed the basal ration developed stones while 54 and 58% of lambs receiving 3/8 and 1/4 in. pellets, respectively, developed stones.

The addition of 1% chloride to the basal ration decreased the incidence from 38 to 25%. Sixty percent of the lambs affected were clinical cases. When 1% potassium chloride was used in the place of sodium chloride, the incidence was reduced to 10% with no clinical cases. These results tend to refute the efficiency of sodium chloride in the control of calculi. Since previous work has indicated that potassium tends to prevent calculi in wethers, potassium chloride could have been expected to offer a measure of protection.

Variations in the dietary mineral intakes of steers were studied at Belts-ville to determine if diets which produce a high incidence of urinary calculi in fattening lambs were equally calculogenic when fed to steers. Steers were fed diets of known mineral composition which contained an excess of phosphorus (.75%) in the presence of high and low calcium (.025 and 1.50%) and high and low potassium (0.35 and 2.00%). These diets were not predisposing to urolithiasis when fed for a 98-day feeding period. Metabolism data including the amount of minerals in the feces and urine and the serum mineral levels are being studied to determine to what extent the mineral metabolism of these animals was affected.

Two studies were made with laboratory rats to investigate the effects of dietary mineral imbalances on the development of urinary calculi. The vari-

ables, expressed as a percent of the synthetic diet, were two levels of calcium, 1.2 and 0.3; two levels of phosphorus, 1.2 and 0.3; three levels of magnesium, 0.5, 0.25, and 0.05; and two levels of potassium, 0.9 and 0.1. Urinary calculi occurred in four of six animals which received the diet that contained .3% calcium, 1.2% phosphorus, .5% magnesium, and .1% potassium. None of the other 23 diets were calculogenic to the animals which were fed for 14 weeks. Certain dietary mineral imbalances appear to give rise to conditions which promote the development of urinary calculi. (AH d2-31)

4. Pesticide residues. In vitro experiments with organophosphate, chlorinated hydrocarbon, and carbamate containing insecticides were tested with bovine rumen ingesta fractions. The main experimental approaches have been (1) bacterial growth responses to the insecticides in incubated rumen fluid, (2) bacterial studies for the detection of bacterial breakdown of the insecticides, (3) manometric studies using washed suspensions of ruminal ciliates, bacteria, and plant debris with insecticides, (4) determination of quantities of volatile fatty acids produced by the ruminal ciliates incubated with insecticides, and (5) the use of radioisotope and colorimetric techniques for the measurement of insecticide uptake by the ruminal ciliates.

Ruminal bacteria exposed to O to 500 ppm. insecticide levels in rumen fluid starch feed extract medium. No apparent significant bacterial population inhibitions were noted with Dimethoate, Diazinon, lindane, Thiodan, Sevin, and Guthion. Warburg manometric experiments showed paraffin oil-Triton X-155 preparations of Dimethoate, Diazinon, lindane, Thiodan, and Sevin stimulated gas production in holotrich protozoa. Entodinium simplex, and oligotrich, produced less gas with Diazinon substrate than did Isotricha sp. Thiodan breakdown experiments with Isotricha were followed colorimetrically and a 15% loss in substrate occurred during short incubation periods. Diazinon-C-14 uptake by E. simplex and I. intestinalis was measured and significant increases in counts were found in the protozoan cells. Ruminal protozoa are useful as bio-assay tools for screening insecticides which are susceptible to microbial breakdown and may be used on forage and other cattle feed crops.

Studies with pesticides that may be ingested by ruminants were conducted by using both in vivo and in vitro methods. Residue studies were continued to determine specific information as to the effects of Thiodan ingestion by beef cattle. Steers were fed Thiodan by mixing the pesticide with the diet. Steers were confined to metabolism stalls for urine and fecal collections. Tissue residues of 0.5 and 1.0 ppm. of Thiodan were found in omental fat samples of steers receiving 1.10 mg. of Thiodan per kg. of body weight for 30 and 60 days, respectively. At this level of Thiodan intake, the amount of the pesticide occurring in the feces and urine was 6.2% of the total daily intake. The low levels of Thiodan found in the omental fat and excreted in the feces and urine indicate that this compound is probably metabolized. Further analyses of tissues in which a residue of Thiodan was found by the colorimetric method are being made by the use of gas chromatography. This method of analysis will provide a technique to determine if possible metabolic products if the pesticides are present. (AH d2-32)

5. Microbiology of the rumen. Research on various phases of the physiology of the ruminal protozoa have been continued. Besides participating in the breakdown of protein and carbohydrate, several species of the protozoa have been shown to contribute to the lipid metabolism of the rumen. Washed suspensions of the ruminal ciliates I. prostoma and E. simplex concentrated C-14 fatty acids such as oleic, stearic and palmitic. Radioautographs demonstrated that oleic acid was hydrogenated to stearic acid by I. prostoma, and Warburg manometric data showed the sodium salts of oleic, valeric and caproic stimulated acid fermentation by I. prostoma. The total lipid and free fatty acid contents of I. prostoma were determined. Volatile fatty acids were produced by I. intestinalis and ruminal bacteria with tributyrin, but not with tripalmitin. Comparison of mixtures of ruminal bacteria from calves harboring either I. intestinalis or E. simplex indicated they were similar in their response to fatty acids when measured manometrically.

Preliminary results with silicic acid columns and organic solvents indicate that approximately 40% of this lipid is a carbohydrate containing phospholipid. Sterol components are present in the <u>Isotricha</u> lipid both in the sterol and the diglyceride (free sterol) fractions. Other preliminary data indicate that <u>Isotricha</u> sp. respond to sterols dissolved in paraffin oil, particularly corticosterone. Hexestrol stimulates less gas production with <u>Isotricha</u> sp. than with corticosterone. <u>Entodinium simplex</u> is stimulated by either corticosterone or androstendione. (AH d2-24, d2-26)

6. Value of high-nitrogen molasses. In metabolism studies in which 40% of the ration was either a hi-N-molasses, molasses plus urea, or molasses plus cottonseed meal, the dry matter and energy were less digestible in the hi-N-molasses ration than in the rations containing urea. Fiber digestibility was less in the hi-N-molasses ration than in the cottonseed meal-molasses ration and crude protein was more digestible in the urea containing rations than in those rations containing no urea.

Results from both metabolism trials indicated there were no ration differences related to nitrogen retention where expressed as grams per day or as a percent of the nitrogen consumed. (AH d2-14)

- 7. Coumestrol feeding. Preliminary studies with the feeding of 0, 1, and 5 gm. of coumestrol to growing steers resulted in a decrease in the apparent digestibility of crude protein by animals consuming coumestrol and an increase in the percentage of nitrogen retained. Average daily gains appeared to be linearly related to coumestrol intake, but when expressed on a shrunk weight basis, there appeared to be no difference. The blood serum albumin to globulin ratio was slightly less for the coumestrol fed animals. (AH d2-8)
- 8. Anatomical and physiological factors affecting digestibility. Fiber utilization by calves being fed fiber per os and via abomasal fistulae is being studied.

Initial studies to determine some of the ruminal factors which influence the rate of salivary secretion have been completed. Large between animal variations were apparent as was a 30 to 40 min. adaptation period during collection of the saliva from the esophageal cannulae. Studies are now in progress with four steers having ruminal and esophageal fistulae (AH d2-8)

B. Concentrates

l. High-concentrate rations for finishing steers. A series of trials using high-concentrate rations for finishing cattle have been completed at Beltsville, Maryland. The basal rations contained ground shelled corn or ground corn and cob meal, soybean meal, salt, steamed bone meal, and vitamin A. These rations were supplemented by high levels of vitamin A and steamed bone meal, urea, mineral buffers, and zinc used alone or in various combinations. The effect of different levels of crude fiber in the rations and the effect of pelleting these rations were determined.

It was found that cattle consumed significantly more corn and cob ration (7.0% crude fiber) than cattle receiving a ground shelled corn ration (3.0% crude fiber); however, the daily gains, feed efficiency, and carcass data were not significantly different. From these data there appears to be no benefit from increasing the crude fiber in the ration by use of corn cobs. The addition of 2,500 U.S.P. units of supplemental vitamin A per pound of ration did not statistically affect the feedlot or carcass data of cattle when compared to cattle receiving 500 U.S.P. units. The lower level seemed adequate in these rations, but there was a slight trend for the cattle to gain faster during the summer months on the high supplemental level. This was not apparent during the winter months. Analysis of liver samples indicated significantly greater storage of vitamin A of cattle consuming greater storage of vitamin A of cattle consuming greater amounts of the vitamin. There appeared to be no benefit from 2 or 5% steamed bone meal over the 1% steamed bone meal present in the basal ration.

A trial was designed to determine the effects of mineral buffers (2.1% potassium carbonate, 2.0% calcium carbonate, and 0.5% magnesium sulphate vs. none) protein sources (urea vs. soybean meal) and zinc level (25 ppm. vs. 125 ppm.) on the performance of cattle consuming a high concentrate ration based around shelled corn. Results indicated no statistically significant differences in gains among groups of cattle. However, there was a trend for lowered gains among groups of cattle consuming the buffered rations. It was found that cattle consuming the rations containing added mineral buffers had significantly lowered carcass grades and less fat over the rib eye than cattle receiving the non-buffered rations. Considerable bloat was encountered by cattle consuming the buffered rations while there was very little in the groups of cattle receiving non-buffered rations.

In a trial at Tifton, Georgia, to determine the value of hay and a complex supplement in high concentrate rations based on snapped corn, steers fed the supplement and ground snapped corn with or without hay had slightly higher

feed consumption, rate of gain, and carcass grade than steers fed the standard ration. The addition of vitamin A to the standard ration appeared to improve slightly feed consumption, gain, and carcass grade. (AH d2-14)

C. Forage Preservation and Utilization

1. Utilization of Coastal Bermudagrass. In a 75-day drylot feeding trial at Tifton, Georgia, steer calves were full fed Coastal Bermudagrass hay, good quality Pensacola Bahia grass hay, and poor quality Pensacola Bahia grass hay. The good quality Bahia hay was cut just after the emergence of seed heads and the poor quality cut just after harvest of the seed crop. The results show that the steers fed the good quality Bahia gained approximately twice as fast as the other groups. The daily Bahia hay consumption was about 20% greater.

Cows wintered on a full feed of Coastal Bermudagrass hay were compared with cows grazing on pasture for 4 or 5 hours per day plus a limited amount of Coastal Bermudagrass hay. The birth weights of calves dropped by the grazing cows were about 7 lb. heavier and the calves gained about 20% faster than those of cows fed hay. The cost of wintering per cow or per day was essentially the same for both treatments. (AH d2-3)

In studies at North Carolina, 64 yearling steers were used to determine the influence of energy and protein supplements on performance and carcass value of cattle grazing nitrated Coastal Bermudagrass. There appeared to be no benefit from adding protein supplement over and above the energy derived from the supplement. Self feeding corn-fat mixtures appeared to have considerable value as a method of enhancing the growth-fattening process of steers being finished on Coastal Bermuda pastures. (AH d2-8)

2. Feeding value of pelleted feeds. At Beltsville physical state-animal behavior studies have indicated that the rate or spread of feed consumption is directly related to body weight of the animal and that the type of ration offered may affect that relationship. This is, the slope of the regression line of rate upon weight for the pelleted high roughage ration was greater than the slope for the same ration when fed as a coarsely ground mixture.

Facilities are now being prepared for studying the effect of light and competition upon the feeding behavior of drylot fed steers.

The feeding value of pelleted feed is being studied at several locations. At Tifton, Georgia, high quality Coastal Bermudagrass pellets were supplemented with a complex mixture which contained protein, molasses, minerals, and vitamin A. In 140-day feeding trial, performance of steers fed a high quality Coastal Bermudagrass pellet was not appreciably affected by replacing 10% of the pellets with the complex supplement. There was a slight improvement in carcass grade and yield in the steers fed the supplement.

Pellets made from dehydrated Coastal Bermudagrass were compared with those made from sun-cured Coastal Bermudagrass. Both types of pellets were supplemented with a complex supplement and with 0, 30, or 60% cracked corn. No consistent differences were noted in steer performance due to type of pellet fed. Adding corn to the ration improved gain, dressing percentage, carcass grade, feed efficiency, and selling price. When properly supplemented, pellets made from good sun-cured Coastal Bermudagrass hay were equal in feeding value to pellets made from dehydrated Coastal Bermudagrass. However, adding corn to either type of pellet is desirable in the fattening process.

In a 140-day feeding trial the value of implanting steers with 30 mg. of diethylstilbestrol was determined when the steers were fed dehydrated or sun-cured Coastal Bermudagrass pellets with 30 or 60% cracked corn plus supplement. On the average, the diethylstilbestrol implants increased gain 14% and reduced feed needed per pound of gain by 11%. No consistent effect was noted on feed consumption, dressing percentage, or carcass grade. (AH d2-28)

3. <u>Limited vs. free choice alfalfa hay.</u> In studies at Fort Robinson, 340 heifers were used to determine the effect of feeding limited amounts of alfalfa hay as compared to free choice feeding on the feedlot performance of yearling heifers.

In the first trial 50 heifers were fed 2 lb. of alfalfa per day and 50 others were permitted to eat all that they would consume, which averaged slightly over 5 lb. per day. The grain ration (80% cracked corn, 15% dried beet pulp, and 5% soybean meal) was self-fed to all lots. Salt and steamed bone meal were fed free choice. The average daily gain and cost per 100 lb. of gain were for the limited and free choice groups, 2.35 lb., \$17.63; and 2.34 lb., \$19.81, respectively.

In the second trial a 4 lb.-per-day level was added to those used in the first trial. The same grain ration was fed. The average daily gain and cost per 100 lb of gain for the 2 lb., 4 lb., and free choice groups were 2.15 lb., \$19.95; 2.50 lb., \$18.75; and 2.37 lb., \$19.70, respectively. (AH d2-21)

In a trial at Jeanerette, Louisiana, weanling heifers were fed rations to compare ground hay (Dallis and native Bermuda), corn silage, and grass silage (oats and rye grass). There was very little or no difference in gain or condition score due to treatment. All groups were given an adequate concentrate supplement. A trial with yearling heifers involved the evaluation of ground grass hay and grass silage. There was only 0.05 lb. difference in daily gain of the heifers and 0.3 of a grade difference in the condition score. These results indicate there is little or no difference in these roughages for wintering heifers. (AH d2-34)

D. Range and Pasture Management

1. Range supplementation studies. A project designed to determine the effect of age at first calving and level of winter feeding of beef cows on the breeding efficiency, longevity, and economic production of calves has been underway since 1948 at Fort Reno, Oklahoma, in cooperation with the Oklahoma Agricultural Experiment Station.

There were 117 heifers at the start of the experiment and the number of cows remaining in the herd and the average percent calf crops were: through 8 years of age, 107 cows, 94.9% born and 90% weaned; through 10 years of age, 99 cows, 94.5% born and 89.5% weaned; through 12 years of age, 81 cows, 93.8% born and 88.6% weaned; through 14 years of age, 57 cows, 91.9% born and 86.9% weaned.

The cows in this study began to decline in reproductive performance at 10 years of age, with a marked decline in cows 12 years of age or older. The decline in the older cows was one of failure to conceive.

There was no adverse effect of two-year-old-calving on the future reproductive performance of the heifers at 8, 10, and 12 years of age, respectively, 21.5%, 28.3%, and 33.3% of the cows had been open at least once.

The practice of culling open cows would have increased the calf crop for the following calving season by about 5% but would not have been effective in reducing the number of open cows in the herd in future years. The different winter levels produced marked differences in several economic traits but there were no consistent differences in any aspect of reproductive performance.

Estimates of milk production on more than 300 range beef cows representing both spring and fall calving herds were analyzed to determine some of the factors involved. There was a wide variation in milk production among individual cows (5.9 to 14.3 lb.). The body size of the cow had little influence on milk production. Milk production is a highly repeatable trait. The feed level prior to and during lactation had a marked influence on milk yields. (AH d2-12)

In an experiment at North Carolina, 40 yearling Hereford steers were used to compare grazing and soilage as methods of furnishing forage to fattening steers fed corn with added fat to limit concentrate intake. Grazing steers gained 0.2 lb. more per day while consuming 40% less concentrate than steers fed green chop. The steers consumed an average of 26 lb. of green chop per head daily. No large differences were observed in carcass characteristics. (AH d2-8)

E. Management Practices

 Management of cattle and pastures for beef production. Creep feeding experiments carried on for 4 years at Brooksville, Florida, with Angus. Hereford, Brahman, and Santa Gertrudis breeds and a Brahman-Angus group showed wide breed differences. The creep-fed calves showed an advantage of 15 lb. over the noncreep-fed calves at weaning. The average increase in weight of the creep-fed over the noncreep-fed was for Angus 24 lb., Hereford 34 lb., Brahman -3 lb., Santa Gertrudis 14 lb., and Brahman-Angus 11 lb. While the creep-fed calves made more gain during the preweaning period, the opposite was true during the post-weaning period. At 18 months of age, the noncreep group averaged 661 lb. while the creep group averaged 652 lb. Thus, by 18 months of age the advantage of creep feeding had disappeared.

In a trial at Brooksville, Florida, 24 steers were full fed on pasture for 210 days, one-half of the steers were given 25,000 U.S.P. units of vitamin A per head daily. The vitamin A steers gained 0.04 lb. per day more and were almost a third grade fatter than the control group. The vitamin A steers had a brighter, cleaner hair coat, and looked better than the steers in the control group. (AH d3-2)

The value of corn silage and limited concentrates for feeding cull beef cows was studied at Jeanerette, Louisiana. Cows receiving silage and concentrates gained in condition from cutter to utility while the non-fed group remained in the same condition. The market value of the fed cows increased about 2 cents per pound. There was no increase in value of the non-fed cows. (AH d2-34)

2. Beef production from beef, dual purpose, and dairy steers. This experiment is being conducted in cooperation with the Dairy Research Branch and Meat Quality Laboratory to study several systems of feeding and management as related to the economy and value of beef from dual purpose and dairy cattle.

The first replication has been completed in which Holstein, Milking Shorthorn, Jersey, and Angus were fed the first six months of life either on a high plane of nutrition involving large quantities of milk or a low plane representative of dairy replacement feeding practices. For the second phase, steers of each breed and previous nutritional plane were subdivided into four groups. These were: (1) slaughtered at six months to determine body composition, (2) high concentrate ration, (3) all-hay ration, or (4) all-hay ration for most of the period followed by a finishing period on the high concentrate ration. Average 180-day weights were 440 and 220 lb. on the high and low planes, respectively. Holsteins gained proportionately faster and Milking Shorthorns slower on the low nutritional plane during the first six months. Holsteins gained most rapidly on all rations in both phases followed by Milking Shorthorn, Angus, and Jerseys. Steers fed at the lower level during the first six months gained more rapidly and more efficiently during the second phase. Tenderness and palatability of the meat, fat content, and lean content were related to the feeding regimes during the second phase. fatness of carcasses the breeds ranked Angus, Milking Shorthorn, Jersey, and Holstein. The Angus ranked highest and Milking Shorthorn lowest in tenderness. The Angus was the most efficient in fat production. The second replication has been started with some modifications in the feeding regimes for the first six months. The Angus were replaced by Herefords for this trial. The milk replacer has been increased 20% and a calf starter is being fed for the first 60 days, then replaced with the calf grain which is fed up to 4 lb. per day. The maximum amount of milk being fed is 30 lb. per day. The average daily gains during the first six months for Herefords, Holsteins, Milking Shorthorns, and Jerseys on milk are 1.91, 2.78, 2.43, and 1.81 while the gains on milk replacer are 1.00, 1.54, 1.34, and 1.14. These rates of gain are higher than those in the first replication. (AH d3-6) (Also see Area 5 c-5)

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INFECTIOUS AND NON-INFECTIOUS DISEASES OF CATTLE Animal Disease and Parasite Research Division, ARS

<u>Problem.</u> Losses from infectious and non-infectious diseases of cattle, other than those due to parasites, are estimated at approximately \$600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA PROGRAM

The <u>Department</u> has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The <u>Federal</u> scientific effort devoted to research in this area totals 57.7 professional man-years. This effort is divided among sub-headings as follows:

Brucellosis, 2.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Universities of Maryland, Minnesota, and Wisconsin.

<u>Vibriosis</u>, 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and <u>under cooperative agreement</u> with the New York State Veterinary College at Ithaca.

Tuberculosis, 6.6 at the National Animal Disease Laboratory, Ames, Iowa, and through two contracts with the Michigan State University, East Lansing.

Mucosal-Respiratory Disease-Complex, 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Indiana (Lafayette) and Iowa (Ames) Experiment Stations, and the Colorado State University (Fort Collins).

Mastitis, 6.2 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Respiratory Disease (Shipping Fever), 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Leptospirosis, 6.0 at the National Animal Disease Laboratory, Ames, Iowa.

Infertility in Cattle, other than vibriosis and trichomoniasis, 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Epizootic Bovine Abortion, 3.4 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Foot Rot, 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

<u>Paratuberculosis (Johne's Disease)</u>, 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Keratitis (Pink Eye), 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Brucellosis

Research workers at the National Animal Disease Laboratory (NADL), Ames, Iowa, reported the pathology of 2 bulls, naturally infected with Brucella abortus, was studied for 5 and 2 years, respectively. Serologic, bacteriologic and histopathologic examinations were correlated with the clinical signs of the disease. Seroagglutinin and semen plasma agglutinin titers persisted at diagnostic levels throughout the study, and Br. abortus was consistently isolated from semen of both bulls. At necropsy, Br. abortus was isolated from the testes, epididymides, seminal vesicles, and the ampullae of the ductus deferens. Pathologic changes were observed throughout the genital tract. Granulomas, including sperm granulomas, were found in the epididymis of one bull.

Modern techniques for processing and distributing semen from such bulls create a situation, wherein thousands of cows may become infected with brucellosis. Semen plasma agglutination tests, seroagglutination tests, and bacteriologic examination of semen offer the best means of detecting an early or clinically inapparent infection.

Recent research has been directed toward differentiation of Brucella agglutinins in bovine serums on the basis of their heat stability at 65 C for 15 minutes. By this criterion a heat labile and a heat stable seroagglutinin have been demonstrated. Ultracentrifugation revealed that the heat labile seroagglutinin had a higher molecular weight than the heat stable seroagglutinin. The heat labile agglutinin was found in the serums of cattle known to be brucellosis-free; in the serums of calves after vaccination with Brucella abortus Strain 19; and in serums of heifers with persistent post-vaccinal seroagglutinin titers. It was also found in the serums of cattle recently exposed to virulent Brucella abortus and in serums of cattle that became infected. The heat stable agglutinin was found only in the serums of cattle that were infected or exposed to Brucella abortus. The heat labile

seroagglutinins were predominant at the onset of the disease, whereas the heat stable seroagglutinins became predominant as infection progressed. Heat stable Brucella seroagglutinins were an indication of exposure to Brucella_abortus, whereas the significance of the heat labile seroagglutinins was not as readily apparent.

Acidified Plate test antigens at pH 4.0, 3.6, 3.4, 3.2, and 3.0 were evaluated in supplementary tests to clarify the status of "suspect" cattle to standard seroagglutination tube and plate tests for bovine brucellosis.

The serologic response of 57 vaccinated and 22 nonvaccinated animals was studied after conjunctival exposure in midgestation to about 7 x 10 cells of virulent <u>Brucella abortus</u> Strain 2308. The brucellosis status of each animal at the termination of pregnancy was determined by bacteriologic examination of blood, milk, uterine contents and fetal organs.

The acidified plate antigens were compared for their inhibitory effect on serologic reactions of infected and noninfected cattle.

Inhibition of the suspect serologic reactions was directly dependent upon the magnitude of the original standard seroagglutination tube or plate test titer and the pH of the antigen. Serologic reactions in the lower suspect titer range were more readily inhibited. The inhibitory effect increased gradually as the final pH of the antigen-serum mixture decreased.

Since low level seroagglutination reactions involving specific Brucella agglutinins were frequently inhibited by the antigens, such antigens have little value in supplemental tests to determine the brucellosis status of "suspects" in an infected or problem herd. (NADL)

The University of Minnesota, under a cooperative agreement with the USDA, continued the studies of macroglobulins of milk and serum, but the emphasis was on developing methods of application of basic findings. Studies during the past year have been concerned with evaluating a new test procedure to detect macroglobulins for Brucella in cattle serums and milk, studying the occurrence and distribution of these agglutinins in a certified county, and studying their appearance and persistence in experimentally infected swine. (Minnesota)

The University of Wisconsin, under a cooperative agreement with the USDA, initiated a systematic study of the complement-fixation test for use as a diagnostic procedure in the research on brucellosis. A standardized method for the test has been developed. Preliminary data indicate that the test is a useful supplemental test for subjects in problem herds. It permits differentiation of infected vaccinated heifers from non-infected vaccinated heifers with persistent agglutinin titers. Other data comparing the CF with other supplemental tests are being analyzed.

In the immunochemical determinations, phenol extracts from <u>Br. abortus</u> have been compared with those previously obtained by some disruption of cells. These have been studied by serological and biological methods. (Wisconsin) (ADP al-3(Rev.)

B. <u>Vibriosis</u>

The National Animal Disease Laboratory (NADL) workers reported their investigations were directed toward analysis of the cellular changes in the uterine endometrium due to \underline{V} . fetus infection. Twelve virgin heifers were infected by natural service to a \underline{V} . fetus-infected bull. At intervals of 8 to 122 days after initial exposure, the heifers were necropsied and uterine sections obtained for histopathological studies. Five unbred heifers and 7 heifers bred to a noninfected bull, were necropsied at intervals of 0 to 39 days after estrus or breeding and uterine sections were obtained for comparative studies as controls.

All heifers bred to the infected bull became infected at first service and microscopic studies of uterine sections revealed neutrophilic and lymphocytic periglandular infiltration of the endometrium that was not observed in non-infected heifers. The presence of a moderate inflammatory process suggested the underlying cause for lack of conception and subsequent pregnancy. Histologic differentiation was not observed in the cervicovaginal areas, cervices, or ovaries of any heifers. Only 2 of 12 infected heifers had evidence of pregnancy when euthanized compared with 6 pregnant of the 7 non-infected heifers. (NADL)

The New York State Veterinary College, Ithaca, under a cooperative agreement with the USDA, continued research investigations on diagnostic procedures for vibriosis. One of the principal difficulties encountered in the control of vibriosis is the diagnosis of the asymptomatic carrier state in the bull. Cultural methods are not reliable enough for routine diagnosis in all bulls. The inoculation of test heifers, with material taken from suspect bulls, is a very expensive and time-consuming procedure. The fluorescent antibody technique provides a promising method which would be much more rapid and much less expensive.

Fluorescein conjugated rabbit gamma globulin against $\underline{\text{Vibrio}}$ $\underline{\text{fetus}}$ has been prepared. It has been tested on pure cultures of $\underline{\text{V}}$. $\underline{\text{fetus}}$ and found to stain these organisms satisfactorily. Hyperimmune gamma globulin has been prepared against other species of vibrio and common contaminants. This will be used to insure specificity.

In the study of the incidence of <u>vibrio</u> fetus carrier bulls, it was found that both age and tenure were found to have a highly significant effect on the rate of infection. The frequency of infection was at a peak during the age range of 6 to 11 years, the most useful life span of AB proven bulls. The incidence of carrier status in bulls that entered the stud as young sires, increased almost five-fold as they entered their 7 to 8-year tenure period. The chance of exposure and infection increases with age and tenure.

Toxicity trials were conducted using nitrofuran drugs for the treatment of infected bulls. Furazolidone cream (1%) and Furacin solution (0.2%), have been tested in bulls at two and three times the usual number of treatment applications in an attempt to determine possible toxic effects. Six bulls (2 controls, 2 given 6 treatments, and 2 given 9 treatments) were used in this trial. Weekly semen samples were collected for 3 weeks prior to the start of the treatments, and were continued on all bulls until 10 weeks after the end of the treatments. No deleterious effects on the penile mucosa, semen quality or health of the bulls were observed. Semen volume, motility, concentration, and morphology were determined on all samples collected. Semen from all bulls remained normal throughout the experiment. (New York) (ADP al-9(Rev.)

C. Tuberculosis

Research was continued at the Michigan State University under two contracts with the USDA. Reports submitted are as follows:

(Contract No. 12-14-100-6852(45). During this year 27 calves which did not react to avian or mammalian tuberculins or Johnin were purchased. The herds of origin of these animals were reported as free of tuberculosis by the U.S. Department of Agriculture. These animals were divided into 9 lots of 3 each. Three lots were infected with a Group III mycobacterium which induced the greatest sensitivity in guinea pigs to mammalian tuberculin: 3 lots with a Group III mycobacterium which induced greatest sensitivity in guinea pigs to avian tuberculin, and 3 lots with a Group III mycobacterium which induced greatest sensitivity in guinea pigs to Edward's purified protein derivative-Battey (PPD-B). Three animals in each of 3 lots were inoculated via the intradermal route; 3 via intrauterine route, and 3 with an aerosol of the organism. Blood samples and tuberculin sensitivity data have been, and are being obtained at appropriate intervals. One animal from each lot has been or will be sacrificed at 2, 4, and 6 months post-inoculation, or earlier, if the animal's condition necessitates. Tissues from each animal were, or will be examined bacteriologically and pathologically to determine the infectivity of the organisms.

Serums collected are being examined for polysaccharide and phosphatide specific antigen. The precipitinogenic relationship of mycobacteria of human and animal origin has been investigated using antiserums produced in rabbits. Selected strains (24) of mycobacteria of human and animal origin have been examined for specific lipids by chromatologic fractionation and infra-red spectrophotometric analysis.

Studies on the change in virulence in selected atypical isolants induced by repeated passage through guinea pigs are in progress.

(Contract No. 12-14-100-5786(45). The first work undertaken on this contract was the testing of samples of feed supplements of animal origin for the presence of acid-fast organisms. To date 107 samples have been examined.

Sixty animals to be used in the study were obtained from a herd in which no tuberculin reactors were found. In addition, the animals purchased had no detectible response to avian or mammalian tuberculins or Johnin. When tested with these products in the cervical region, the animals were transported to and are maintained in the barn used for the study in such a way as to prevent their contamination by other animals.

The work has progressed according to the schedule presented in the first semi-annual progress report of this project. More specifically, the animals were first tested for the presence of internal parasites, leptospirosis, and brucellosis. Then they were tuberculin tested, using mammalian tuberculin in the caudal fold. On February 13, the feeding of the experimental ration was started. Group A (15 animals) were fed the control ration; Group B (15 animals) the control ration with killed Mycobacterium bovis added; Group C (15 animals) the control ration with killed Mycobacterium avium added, and Group D the ration containing meat and bone scrap and steamed bone meal as detailed in the previous report.

Caudal fold tuberculin tests using mammalian tuberculin were performed on different lots of 5 animals of each group at 20, 30, and 40 days following the start of feeding the experimental rations. All animals were tested with mammalian tuberculin injected into the caudal fold after being fed the experimental rations for 100 days. (Michigan) (ADP al-13(Rev.)

D. Mucosal-Respiratory Disease-Complex

Research studies were continued at the National Animal Disease Laboratory. A soluble antigen present in infectious tissue culture fluids was separated from the infective virus particle by ultracentrifugation of two serologically related strains of bovine viral diarrhea viruses, NADL-MD and Oregon C24V.

Neutralizing antibodies against the two viruses were absent in four hog cholera antisera, but present in significant titer in a commercially prepared antiserum. Precipitin tests utilizing the agar double diffusion technique formed a single line of identity between the concentrated soluble antigen of both viruses and NADL-MD and hog cholera antisera. No lines were observed using concentrated virus pellet, noninfected embryonic bovine kidney cell antigens, specific pathogen-free calf serum or swine sera.

The relationship between the antigens of bovine viral diarrhea and hog cholera were investigated cooperatively with the Hog Cholera Project. Specific staining of antigen within bovine embryo kidney tissue culture cells, infected with either Oregon C24V or NADL-MD bovine viral diarrhea virus, was accomplished using fluorescein-conjugated swine anti-hog cholera or bovine anti-viral diarrhea globulin. Also specific staining of antigen within pig kidney tissue culture cells, infected with hog cholera virus, was accomplished using the same two types of conjugates. Specificity was confirmed by appropriate controls.

It was found that immunofluorescence was a convenient and sensitive method for determining an antigenic relationship between hog cholera and bovine viral diarrhea viruses. (NADL)

Colorado State University, Fort Collins, under a cooperative agreement with the USDA, made investigations which were reported as follows: Studies were conducted on the longevity of immunity to infectious bovine rhinotracheitis (IBR), presently considered in the Mucosal-Respiratory Disease-Complex. During the past year, the serum neutralization titers of cattle which are kept in the isolation units did not show lowering of titer. There was no difference in serum titers between the group which was infected intratracheally and the group which was infected intramuscularly.

In studies on the susceptibility of mule deer to IBR, 18 of 50 deer, obtained from different areas in Colorado, were found to have significant antibody titer of IBR, 12 of them had equivocal titers and 20 of the deer were free of IBR titer. Twelve of the 20 deer were used for testing the susceptibility of IBR. These deer with significant titer of IBR were also challenged with IBR virus to see whether it would produce any clinical reactions. The results were negative. In addition to the deer, 3 elk and 1 antelope were also inoculated with IBR virus, but they did not show any clinical reactions.

Two to 4 days after injection of IBR virus intratracheally, clinical reactions were shown among the deer. Generally they showed anorexia, depression, excess salivation and respiratory distress such as increased respiration rate, dyspnea and occasionally dry cough. Two of the deer also showed excess serous nasal discharge. One deer showed conjuncto-keratitis 4 weeks after infection at which time the animal was normal otherwise. All the clinical signs were milder than those of cattle. Hematological values of white blood cells and differential counts were within the normal range.

Virus was isolated from the nasal secretions for 7 days after inoculation of virus. Virus was also isolated from the deer with conjuncto-keratitis from the eye swab. One virus isolation was obtained from the fecal swabs from another deer. This virus isolated from the fecal swabs was obtained from a deer also 4 weeks after injection of virus while there was no sign of sickness which could be observed. No specific clinical reactions were observed after challenge, which was 5 weeks after initial infection. There was no death loss resulting from IBR infection.

The pattern of serological response was similar to that of cattle. The measurable antibody titer appears 5-7 days after infection. It took 10-12 days to reach the height of the antibody level of $10^4 \cdot 5$. The results obtained show evidence that deer are susceptible to and play a role in the epizootic of IBR.

In a study of the pathology of IBR in relation to abortion, five groups of pregnant cows, with 5 head for each group, were used for pathological and virological studies. Ten additional cattle were used as controls.

Group	No. of cattle	Stage of pregnancy	Time between infection and fetal material collected
I	10	lst-3rd trimeste	r
II	5	lst trimester	3½-5 weeks*
III	5	1st trimester	4 weeks
IV	5	3rd trimester	2 weeks
V	5	3rd trimester	3-4 weeks*
VI	5	lst trimester	l week

^{*}Two abortions occurred after infection and 1 dead fetus found in uterus.

The abortion occurred in both first and third trimester pregnancy. The approximate time for producing abortion after infection with IBR virus in pregnant cows is approximately 3-5 weeks. Due to the spontaneous abortions in Group II and V, the rest of the pregnant cows in those groups were sacrificed, so that the specific pathological changes could be traced. During this process, one dead fetus was found from each group which gave more convincing evidence that the fetal materials were obtained close to the abortion. Materials collected are being prepared for pathological study and virus isolation. (Colorado)

Purdue University, Lafayette, Indiana, under a cooperative agreement with the USDA, reported sporadic cases of acute virus diarrhea and mucosal disease continue to occur in Indiana. Serums obtained from such herds contain high titers of neutralizing antibody against the C24v strain of virus.

Typical cases of experimental virus diarrhea followed the inoculation of susceptible calves with tissues from two of three field cases of suspected virus diarrhea. In addition to demonstrating the current presence of active virus in the herds of origin these trials provided two new isolates of virus diarrhea agent which will be tested for immunologic relationships with known strains and possible cytopathogenicity.

An agent which is cytopathogenic for ovine thyroid cell cultures was propagated in these cells during the serial passage of VD 46 virus. Subsequent study suggests that the cytopathogenic agent is not VD 46 virus or a bacterium. The identity of this (viral?) agent has not been established.

A metabolic inhibition test for the assay of polio virus was adapted to the assaying of VD-MD viruses. Virus effects were noted only when undiluted and 10⁻¹ dilutions of virus were employed and so the sensitivity of the assay under the conditions employed was judged to be impracticable.

The development of a passive hemagglutination test for the detection of antibodies against VD-MD was attempted for its obvious advantage as a diagnostic test and as an aid to laboratory study of this disease complex (cross protection tests in animals and neutralization tests in tissue culture are definitive but costly and time-consuming). The test system developed employs C24v virus grown in bovine embryonic kidney cell cultures and tanned and labelled sheep red blood cells. Thus far only low titer agglutination reactions have been observed with selected field sera and with experimental sera with cells labelled with concentrated soluble antigens. However, these low titer reactions were observed only in post-inoculation samples, so studies are in progress utilizing other strains of VD-MD virus and a wider range of experimental and field case serums.

Paranatal hematologic values for Caesarean-derived calves are reported. The major developmental changes appeared to be complete by weeks 8 to 10. The lack of immature neutrophils at weeks 8, 10, and 12 indicated a relative freedom from bacterial infections. Total serum proteins increased throughout the first 12 weeks with marked changes in the relative percentages of the globulin components.

Birth weight, 180-day weaning and yearling weights with average daily gains are presented for the SPF cattle herd. This limited data indicates that the major differences in this regard, between Caesarian-derived and second generation calves, occurs prior to weaning.

A bacteriological survey directed to the detection of selected bacterial pathogens was conducted in the SPF cattle herds. Forty animals were evaluated in March, 38 in April, and 32 in June. Three specimens were taken from each animal each month - nasal swabs, eye swabs, and fecal specimens. Large numbers of several species of bacteria were isolated but during the period of this survey none of the animals appeared to be harboring bacteria known to be capable of inducing disease in cattle. This appeared to be true even though herds on surrounding farms continued to present a history of diseases associated with the specific bacteria sought in this survey. (Indiana)

Iowa State University, Ames, under a cooperative agreement with the USDA, reported work on a new serological strain of virus diarrhea virus.

Identification: The new strain of virus diarrhea virus referred to as MDI-2 was a distinct serotype of virus diarrhea. The MDI-2 strain, however, does share a common soluble antigen with other wirus diarrhea strains. This common antigen can be detected by the fluorescent antibody technique.

It is evident that the new strain is serologically distinct because of three reasons - 1) reciprocal cross protection tests with MDI-2 and other virus diarrhea agents failed to demonstrate cross protection: 2) specific rabbit prepared antisera to the MDI-2 agent and to C-24-V virus prototype failed to show reciprocal cross neutralization, and 3) vaccination with MDI-2 failed to protect pigs from a subsequent challenge with hog cholera virus.

Characterization: Production of Disease in Calves: MDI-2 produces a very distinct experimental disease in calves inoculated intravenously. The reaction is characterized by a diphasic temperature response with a concomitant leucopenia associated with the primary temperature elevation. Clinical signs of anorexia, dyspnea, diarrhea, increased lachrymation, and salivation were present.

Properties of the Virus:

- 1. Structure and classification: Electron microscopic studies revealed that the virus has a structure and a developmental cycle identical to that of myxoviruses in general. Since our antigenic studies revealed that the MDI-2 agent is related to other strains, we now conclude that the entire group of virus diarrhea agents are myxoviruses.
- 2. Ether sensitivity: The MDI-2 agent is ether sensitive which would support the fact that it is a myxovirus since all members of this group are ether sensitive.
- 3. Antigenic characteristics: We have demonstrated by a combination of serum neutralization trials, fluorescent antibody tests, and double diffusion studies in agar, that two viral antigens exist. V soluble antigen appears to be responsible for cross fluorescense studies with other agents which are not related insofar as they do not cross neutralize. The soluble antigen will react with anti C-24-V antiserum in a double diffusion test.
- 4. Growth characteristics: MDI-2 virus adsorbs to testicular cell monolayers rather slowly. A 3-hour adsorption time was necessary to insure 98 percent adsorption. By fluorescent antibody studies, we have determined that the first antigens appear within the nucleus at 3 hours. By electron-microscopy, these antigens may be identified as the nucleocapsid which most probably contains ribonucleic acid and protein. The fluorescence gradually disappears from the cell nucleus and appears in the cytoplasm, although there is a period where both nuclear and cytoplasmic fluorescense is seen. The intense fluorescense observed at 10 hours is correlated with densely packed complete virus particles adherent to the cell surface as observed through the electron microscope.

Virus penetration is passive in that the virus particles are apparently taken in by the normal pinocytotic process. The viral envelope is intimately attached to the plasma membrane lining the vesicle and is apparently broken

down within the vesicle. All of these events could be observed in electronphotomicrographs.

It is obvious both from fluorescent antibody studies and electron microscopy that the virus is held at the surface of the cell prior to release which is typical of myxoviruses in general.

This work has presented some very important considerations both from an applied aspect and from a very basic concept. Since this agent has been shown to be a new serological strain of virus diarrhea, it follows that the existing vaccines which only incorporate one virus would not fully protect animals against virus diarrhea.

From a basic aspect it was shown that the MDI-2 agent is related to other virus diarrhea strains by a common soluble antigen. We have also shown that this cross reaction does not exist with the noncytopathogenic virus diarrhea strains. This brings up the interesting point as to the mechanism of cell-killing by the virus. It may be that the presence of the soluble antigen actually kills the cells and that the absence of it is correlated with the noncytopathogenic strains. This idea does have a practical side because of the fact that MDI-2 strain actually is more virulent than the noncytopathic Sanders Agent.

<u>Viral Isolation Attempts from Cattle</u>: It was shown experimentally that the MDI-2 agent produces signs referable to both the digestive tract and the upper respiratory tract. This agent was reisolated from a nasal swab taken from an experimentally infected animal. Attempts to isolate agents from herds clinically affected with mucosal disease were negative. These included isolation attempts from 78 animals. Efforts to isolate agents from three "normal" herds were successful. The agents are being studied.

Pathology of experimental virus diarrhea: A comparative study of the pathology produced by 6 mucosal disease-viral diarrhea agents in calves under experimental conditions has been described. Four of the agents produced similar lesions in the digestive mucosa and lymph tissues which corresponded to the early lesions reported for field cases of mucosal disease-viral diarrhea. Two of the agents, which later were identified as strains of infectious bovine rhinotracheitis virus, produced similar but not as severe lesions as the other 4 agents. In addition, these agents produced multiple foci of necrosis in the adrenal cortex.

Considering the similarity of the clinical syndrome, clinical pathology, immunological protection and lesions produced, it would appear that 4 of the agents studied are closely related. The other 2 agents also appear to be related to each other but not to the other four. (Iowa) (ADP al-14(c)(Rev.)

E. Mastitis

The work conducted at the National Animal Disease Laboratory, Ames, Iowa, was reported as follows: Twenty-five dairy cows with udders free of hemolytic, coagulase-positive staphylococcic infections were tested for blood serum alpha and beta antitoxins for periods up to 2 years. The number of serums positive for these antitoxins and the average antitoxin titers of the positive serums increased with the age of the animals and reached maximum levels during the second lactation period. However, a progressive increase in antitoxin titers during the test period was not apparent when animals were considered individually. In most animals, the titers developed to certain levels and remained relatively stable or declined to levels below 1 international unit (I.U.) of antitoxin.

Three laboratory strains of <u>Streptococcus pyogenes</u> were cultured by daily transfer in a medium composed of 22 amino acids, 2 purines, 1 pyrimidine, B vitamins, inorganic salts and buffered with O.1 M phosphate buffer, pH 7.0. The basic medium is that described by Williams, Cornell Exp. Sta. Bull. No. 337 (1955). It was modified by the addition of L-glutamine, ammonium acetate and O.1 M phosphate. The extra buffer increased growth more than 2-fold. Daily transfers of a 1 percent inoculum resulted in good growth. Optical densities measured in 24 hours with a Klett-Summerson colorimeter and 550 mm filter were 0.40-0.50. One percent glucose was completely fermented to quantitative yields of lactic acid. (NADL)

At the University of California, Davis, under a cooperative agreement with the USDA, a strain of bacteria called <u>Aerobacter aerogenes</u> has been employed which may occur in the environment of dairy cattle. It was selected because it can be readily identified and at the same time will serve as a representative of the fecal (manure) bacteria. Such bacteria, called coliform organisms, are not commonly disease-producers but under certain circumstances of commercial dairying, may enter the mammary gland and produce a very severe inflammatory disease which may lead to death of the cow.

Investigations at California have shown that severe disease results only when a large bacterial population has been produced within the mammary gland. The effects on the cow are not directly related to bacterial growth but rather to destruction of the organisms by the defenses of the body. During multiplication of the organism in the mammary gland, there are no outward signs of the disease, but in time, a reaction sets in (inflammatory) which immediately destroys the organism in large numbers thereby releasing a poisonous substance in high concentration (endotoxin); it is this material released from the bacteria which produces peracute mastitis and which may even lead to death of the cow.

The production of a mild irritation of the mammary gland prior to exposure to the coliform bacteria prevented growth of the organism and the development of severe mastitis. The most important factor in a response to irritation

to mammary tissue is an infiltration of cells (leukocytes) from the blood. It was shown that cells infiltrating into the milk at levels generally accepted as representative of high normal values (250,000 to 500,000 per cc) were capable of inhibiting growth of coliform organisms within the udder and therefore were highly protective against coliform mastitis. The practical implications are that as cows become older and their mammary tissues respond to oft-repeated irritation inherent in the milking process, the leukocyte activity so engendered also at the same time protects the cows against coliform organisms. Therefore, extensive use of antibiotics to reduce mild inflammatory reactions would appear to be inadvisable. (California) (ADP al-15(R)

F. Epizootic Bovine Abortion

The University of California, Davis, under a cooperative agreement with the USDA, reported that during the past year the following findings were made in studies of epizootic bovine abortion (EBA): 1) ingestion, as hitherto believed, does not appear to be the manner in which the virus gains entrance to the body under field conditions: 2) cattle do not become refractory to abortion following exposure, as virgin heifers, to virulent virus: 3) inactivated EBA virus vaccines do not protect cattle against abortion, and 4) the EBA virus appears to be identical with, or closely related to, the virus of enzootic abortion of ewes (EAE).

These findings suggest that the EBA virus is venereally transmitted and an infection immunity develops following exposure. However, it does not develop rapidly enough to prevent abortion in the initial pregnancy. Thereafter, cattle are refractory to reinfection and abortion with this virus because the infection immunity becomes fully operative after termination of the initial pregnancy. (California) (ADP al-21)

G. Paratuberculosis (Johne's Disease)

Research workers at the National Animal Disease Laboratory, Ames, reported that studies were conducted to devise an improved technique for primary isolation of Mycobacterium paratuberculosis. Trypsin digestion of infected intestinal mucosa, followed by decontamination with IN NaOH, was effective in preparing the inoculum for primary cultivation. A lymph node-egg yolk medium was superior to several other mediums for primary cultivation and subcultivation of newly isolated strains.

A herd of cattle, ranging in size from 161 to 195 head, in which Johne's disease was known to exist, was tested periodically with intradermic johnin. Selected tissues of all animals removed from the herd were examined after slaughter for Mycobacterium paratuberculosis. The following observations were made on 96 animals eliminated from the herd during this 5-year study:

Forty-six cattle reacted to intradermic johnin. Fifteen of the reactors developed clinical evidence of Johne's disease, and 21, including these 15, were found to be harboring \underline{M} . paratuberculosis after slaughter.

Twenty of 50 nonreactors were also found to be harboring the bacillus after slaughter, and 10 of these had developed clinical evidence of Johne's disease; a total of 20 cattle, including these 10, were found to be harboring M. paratuberculosis.

Twenty-six cattle that reacted to intradermic johnin were tested periodically for several years, and the following observations were made: a) sensitivity persisted for only 6 months in 12 cattle, 3 of which were found to be infected with M. paratuberculosis when examined after slaughter: b) Sensitivity persisted for 12 months in 5 others, 1 of which was found to be infected when examined after slaughter: c) sensitivity persisted for 36 months in 5 cattle, 3 of which developed clinical evidence of Johne's disease: d) sensitivity was intermittent with no particular pattern in 4 cattle. One of these animals developed clinical evidence of Johne's disease, but in the others there was no evidence of the disease when examined after slaughter. (NADL) (ADP al-35)

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FOOT-AND-MOUTH AND OTHER EXOTIC INFECTIOUS DISEASES OF CATTLE Animal Disease and Parasite Research Division, ARS

Problem. Responsibility for protection of the Nation's livestock industry against diseases, including those of foreign origin, was delegated to the USDA in 1884. Thereafter, contagious bovine pleuropneumonia eventually was eradicated from the United States, thus reopening European markets for American cattle. Ever since then the Department has successively imposed practicable, scientifically justified barriers against introduction of such dangerous exotic diseases as foot-and-mouth disease and rinderpest. The Plum Island Animal Disease Laboratory was established for scientific support of measures for protection against these and other foreign diseases of animals, following the direct threats of spread of foot-and-mouth disease from Mexico and Canada (1946-1954). Foot-and-mouth disease, which is capable of reducing overall productivity by 25 percent in areas where it becomes established, persists in most major livestock-producing countries, except Central and North America, Australia, and New Zealand. Rinderpest continues to be a serious disease problem in Africa and Asia; it is capable of killing 90 percent or more of the cattle that are exposed to it. Other diseases, such as contagious bovine pleuropmeumonia, Rift Valley fever and East Coast fever continue to exact severe tolls in other parts of the world. Possibilities of entry of these diseases into the United States continue, despite all precautions, primarily because of the progressively increasing scope, speed and extent of modern international transportation. The purposes of the Plum Island laboratory are development of basic information applicable to protection of the Nation's livestock from foreign animal diseases; development and maintenance of competence in diagnosis of these diseases, and fundamental research on the biological, chemical and physical properties of the infectious agents that may be useful in prevention, control and eradication of these diseases.

USDA PROGRAM

The Department has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. All of this research is being conducted on the following diseases at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York, except for supplemental field studies on vaccines in The Netherlands.

The <u>Federal</u> scientific effort devoted to research in this area, conducted solely at the Plum Island Animal Disease Laboratory, totals 25.0 professional man-years. This effort is divided among sub-headings as follows:

Pathology -- foot-and-mouth and other exotic diseases 1.0

Fluorescent antibody technique to locate viruses 1.0

Studies of foot-and-mouth disease vaccine 4.0

Immunological investigations to determine the mechanism of antibody formation using viruses of exotic animal diseases 0.5

Immune response to types and sub-types of foot-and-mouth disease virus 1.5

Quantity production of foot-and-mouth disease virus 2.0

Microcinematography of infected cells 0.5

Establishment and characterization of cell lines and cell strains 1.5

Interaction between foot-and-mouth disease virus and host cells 1.0

Genetic biochemistry of foot-and-mouth disease and other exota viruses 1.0

Effects of natural and artificial stresses on foot-and-mouth disease virus 1.0

Bulk freeze-drying of foot-and-mouth disease virus vaccines and antiserums 1.0

Rinderpest of cattle 2.5

Survival and transmission of foot-and-mouth disease virus in semen 1.5

Identification, purification, characterization of foot-and-mouth disease virus 2.0

Immuno-chemical investigations of foot-and-mouth disease 1.0

Survival and inactivation of foot-and-mouth disease virus in meat and meat by-products 1.0

Biological mechanisms of retural resistance and susceptibility to foot-andmouth disease virus 1.0

Work was continued under a PL 480 grant to the Biological Institute, Sao Paulo, Brazil, for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus, and experimental field vaccination.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Pathology - foot-and-mouth and other exotic diseases.

The lingual pathology produced by the virus of foot-and-mouth disease was studied in 165 specimens using a technique designed to record and correlate gross, subgross, and microscopic characteristics of large numbers of lesions. The essential pathological alterations consisted of necrosis of epithelial cells in the stratum spinosum, intercellular edema and granulocytic infiltration. These changes caused development of circumscribed, slightly elevated blanched areas in the linqual mucosa, to which the designation "initial lesion" was applied. A considerable proportion of initial lesions developed promptly into full blown vesicles by separation of the diseased mucosa from the underlying tissue, and the fluid was probably lost through cracks in the stratum corneum. In such areas the desiccating, necrotic mucosa became discolored and the lesion had the gross appearance of a necrotizing rather than a vesicular process. This peculiarity of lesion development was attributed to firm attachment of the thick bovine lingual mucosa by the numerous, well-developed conical papillae.

Lesions in the interdigital skin were basically similar in their initial development but failure to vesiculate was exceptional. In this area, the necrotic edematous skin pulled away easily from the dermal papillae and large vesicles developed. The stress and motion to which the interdigital skin is subject was probably an important contributing factor. (ADP a8-1(R).

B. Fluorescent Antibody Technique to Locate Viruses.

An indirect fluorescent antibody (FA) technique for foot-and-mouth (FMD) evaluation of cattle serums was studied. The reacting system included commercial fluorescein-conjugated rabbit anti-bovine globulin, calf kidney cell cultures infected with foot-and-mouth disease virus (FMDV), rhodamine bovine albumin and test cattle serum. Serums from 55 cattle were evaluated for FA reaction and the results were related to FMD virus experiences of the animals and to their serum neutralization indices.

Serums from cattle that had developed lesions of FMD consistently gave positive or (in two instances) suspicious FA reactions. The different types of FMDV could not be distinguished one from another by the FA reaction when serums of cattle infected with the 7 types were used. The FA reaction was detectable in serums from two steers as early as 6 days and in the serum of one steer as late as 2 years after inoculation with FMDV. One serum tested two years after inoculation gave a negative FA reaction.

Serums from cattle that had not developed lesions of FMD consistently gave negative FA reactions. This included cattle in the following groups: normal controls, convalescing from vesicular stomatitis and virus diarrhea, and animals immunized with an experimental vaccine. (ADP a8-2(R)

C. Studies of Foot-and-Mouth Disease Vaccine.

The efficacy of swine, bovine, and baby hamster kidney cell cultures for the isolation, growth and assay of foot-and-mouth disease virus has been determined. These studies were performed with virus newly isolated from infected cattle. Swine kidney cells were preferable to bovine cells for virus growth and assay when low passage virus was used. Baby hamster kidney cells were very satisfactory and have the advantage that they can be produced in continuous culture. This cell line should prove to be a valuable asset in vaccine investigations.

Studies on the inactivation of foot-and-mouth disease virus indicate that formaldehyde does not reliably kill the virus. The more sensitive cattle tongue inoculation procedure for detecting possible residual live virus in formaldehyde treated preparations revealed that, while tissue culture and suckling mice tests gave no evidence of live virus, the cattle test readily demonstrated it. This more sensitive test thus indicated that formaldehyde was of questionable value for preparation of vaccines of assured safety. The compound, acetyl-ethylene-imine was used to prepare a lot of vaccine that did not infect cattle. This preparation is under potency evaluation. Other inactivating agents will also be examined to determine their possible use for producing vaccines.

Studies to establish the relative immunizing value of different vaccine preparations have been performed. Small animals, e.g., guinea pigs, are being used to circumvent the expense and difficulty of performing such tests in cattle. These studies revealed that the antibody produced by guinea pigs in response to the vaccine changed in its physical, chemical and serological characteristics with time following inoculation. As a result, interpretation of the results will be difficult until the significance of the two different types of antibodies produced is established.

Cooperative research on the extent of immunity of foot-and-mouth disease vaccine conducted in Amsterdam, Holland, revealed that knowledge of the extent and duration of immunity following vaccination against FMD is essential for the proper evaluation of vaccines and scheduling of field vaccinations. Because large numbers of animals are required over long periods of time, such studies can best be pursued in areas where the disease is enzootic and where field vaccination is routinely practiced. Studies in Holland in cooperation with The Netherlands Ministry of Agriculture have continued toward the evaluation of immunity of cattle vaccinated and held under field conditions. Twelve herds, consisting of approximately 400 cattle, are included in this study. Serum antibody levels against type 0 and A foot-and-mouth disease of animals vaccinated two or more times remain high over a 2-year period. Eighty per cent of those cattle which had received several annual field vaccinations and which were challenged 16-48 months later with virulent FMDV, showed resistance to the disease. In general there appears

to be a good correlation between serum antibody level and immunity, and studies will continue to further evaluate and define this relationship. (ADP a8-8(R)

D. Immune Response to Types and Sub-types of Foot-and-Mouth Disease Virus.

Cattle infected with foot-and-mouth disease continue to have virus neutralizing substances, i.e., antibodies, in their blood serum for at least five years after infection. Of three animals kept for this period following infection, one animal retained the ability to resist infection when reexposed to the virus. Another group of cattle studied also demonstrated that antibody is present in sufficient amount for extended periods following infection. This was revealed by precipitation reactions performed by the agar gel method. It was also found that antibodies produced by cattle early in the course of an infection are different than those produced later.

It has been found that calves born of immunized dams do not have antibody to the virus in their serum; however, within 2 hours after receiving colostrum, antibody is found to be present. The transfer of antibody from dam to the calf's serum may be blocked by feeding skim milk or other proteins to the calf before it is allowed to receive colostrum from the vaccinated dam. It was not possible to immunize calves having moderate level of antibody circulating in their blood. It was necessary to let the colostrum-obtained antibody reach low level before satisfactory active immunization could be accomplished. This information is of importance in areas where foot-and-mouth disease occurs, as it is necessary to know at what age calves should be immunized for satisfactory protection. (ADP a8-11(R)

E. Quantity Production of Foot-and-Mouth Disease Virus.

A scheme for rapid modification of foot-and-mouth disease virus (FMDV) populations was developed by growth of the virus in progeny of tissue culture cells that survived infection. Plaque size diminished rapidly as virus was maintained in these cells, and, eventually, visible plaques were not produced. As modification of the virus population progressed, pathogenicity for mice and steers decreased with retention by the virus of significant levels of immunogenicity.

Some basic aspects of FMDV inactivation by glycidaldehyde (GDA) were investigated. The minimal concentration required to inactivate high concentrations of FMDV was between 0.008 and 0.12% GDA. The time required for inactivation of the virus was 105 minutes.

A major part of the effort devoted to this line project was spent in instructing and advising scientists in foreign countries on the growth of FMDV by tissue culture methods. Three months were spent in Turkey at the request of the United States Agency for International Development and the Government of Turkey. During this time a laboratory, consisting of 14 rooms was designed, established, and equipped with laboratory furniture, carts,

trucks, etc. More than 200 different laboratory items were ordered - mostly from the United States. Advice was given on organization of the laboratory and on production procedures.

One month was spent at the Razi Institute in Iran with personnel who requested advice on large-scale tissue culture media production.

Three weeks of instruction were provided at the Plum Island Animal Disease Laboratory to two scientists from Italy on production of FMDV by tissue culture methods. (ADP a8-12(R)

F. Microcinematography of Infected Cells.

Two black and white prints of each of the following films have been prepared:

Cytology I	-18676S	Cell Survival and Cell Culture Regeneration		
		after Infection with Vesicular Stomatitis		
		Virus - New Jersey Type.		

Cytology II -18675S Cell Survival and Cell Culture Regeneration after Infection with FMDV-All9-BK8.

Cytology III -18677S Cell Survival and C 11 Culture Regeneration after Infection with FMDV-All9-PB106.

Cytology IV -18674S Cytopathic Effect of Rinderpest Virus in Tissue Culture.

The following films (reversal film) have been prepared:

Cytology V -18673S Cytopathic Effect of Rinderpest Virus in Tissue Culture.

Cytology VI -18672S Cytopathic Effect of FMDV in Lamb Testicular Cells.

Titles will be redone and prints made of the last two films. (ADP a8-13(R)

G. Establishment and Characterization of Cell Lines and Cell Strains.

A lamb testis cell line developed at the Plum Island Animal Disease Laboratory, was used in microcinematographic study of cellular reactions to infection with foot-and-mouth disease virus (FMDV). This lamb testis cell line was also used in the development of fluorescent antibody technique to locate FMDV in the cell. (ADP a8-14(R)

H. Interaction Between Foot-and-Mouth Disease Virus and Host Cells.

Analytical ultracentrifugation was used to assess the efficacy of physical and chemical separations of 7S and 19S antibodies in sera of guinea pigs

and cattle convalescing from foot-and-mouth disease. A one-step preparative ultracentrifugal procedure separated the two antibody classes. The precipitin reaction was used to determine antibody stability in several solvents.

Foot-and-mouth disease virus was labeled with $P^{32}O_4$ and leucine-3,4-H³. Three classes of RNA with sedimentation coefficients (s₂₀, w) of about 4S, 12S and 23S have been isolated from bovine-kidney culture cells.

A stable baby hamster kidney cell line, obtained from the University of Glasgow, was grown in inexpensive media in roller bottles to populations of about 625 million cells per bottle in 6 days without fluid change. Infection with FMDV yielded fluids containing 10° to 10° PFU/ml. It appears feasible to scale the method upward to produce many milligrams of virus during a single week.

Suckling mice which survive one inoculation with high dilutions of FMDV which still contain appreciable numbers of physical virus particles die when reinoculated 3 to 4 days later with the same statistics as control animals. This suggests that not all FMDV physical particles are lethal and that only those mice die which receive a lethal particle amongst the many administered. (ADP a8-17(R)

I. Genetic Biochemistry of Foot-and-Mouth Disease and Other Exotic Viruses.

Heat denaturation of RNA obtained from virtually pure FMDV by phenol treatment was indicative of pure single-stranded RNA. Its temperature of half-melting (T) in 0.02 M and 0.05 M sodium phosphate at pH 7.5 was 55° and 59°C, respectively. Such RNA contained guanine, adenine, cytosine and uracil in the molar fractions 0.24, 0.26, 0.28 and 0.22. RNA within FMDV did not heat denature until after its release from the protein coat. In 0.05 M sodium phosphate at pH 7.5 this commenced abruptly at 54°C. The T was 70°C. Redenatured virus melted very similarly to phenol-derived RNA with a T of 59°C.

Guanidine reversibly decreased FMDV production in bovine kidney cultures. This inhibition occurred during the latter stages of virus maturation and was not reversed by arginine or urea, both of which are structurally related to guanidine. Virus adsorption by cells was not affected. Para fluorophenylalanine inhibited virus reduplication in cells grown in serum-free medium, but not when serum was present. 2,6-diamino-3 phenyl-azopyridine hydrochloride (Pyridacil) reduced virus production possibly through its cellular toxicity. (ADP a8-18(R)

J. Effects of Natural and Artificial Stresses on Foot-and-Mouth Disease Virus.

Trichlorofluoroethane (TTE) and chloroform are each capable of reactivating virus from a neutral mixture of virus and specific antiserum. One part of

the chemical TTE was mixed with two parts of the virus-serum mixture. The material was then centrifuged and the aqueous layer recovered. Such treatment after eight extractions yielded the maximum virus in the aqueous layer. Butanol was found to inhibit the action of TTE or chloroform to extract the virus from a neutral mixture.

Two subcultures of type A, strain 119 FMDV adapted to tissue culture were compared for stability. After 90 passages, the two lines differed when exposed to 60 C; one was inactivated in 60 minutes and the other in 30 minutes. The two subcultures differed in response to drying on slides at 37C and 20% relative humidity; one survived over 120 days while the other was inactivated in 35 days. Both sublines of virus reacted alike to ultraviolet irradiation and were neutralized by the same antiserum.

Under the conditions of testing, the presence of phenol red in the virus medium did not modify the inactivation time of the virus by ultra-violet light. However, centrifugation at 10,000 r.p.m. for 15 minutes and subsequent exposure of the supernatant to ultraviolet light showed the virus in such preparations to be inactive after 2-hours treatment. Specimens not centrifuged had three logs of virus remaining after 3 hours exposure to ultraviolet light. Twenty ml volumes of virus suspension in a petri dish were used and the fluid film was 0.5 cm thick at a distance of 8 inches below the germicidal light. The intensity was 7/u watts/sq.cm. Tongue tissue suspensions of virus were centrifuged and exposed to UV under the same conditions as the tissue culture virus with the result that 2.5 logs of viral activity remained after 105 minutes of exposure to UV. (ADP a8-19(R)

K. Bulk Freeze-Drying of Foot-and-Mouth Disease Virus, Vaccines, and Antiserums.

Type A, strain 119 foot-and-mouth disease virus, dried in tissue culture fluid in 250 ml amounts at either chamber temperature or with a 37C heat input to the drying chamber, did not process or store well at 4, 23, or 37C for 3, 2 or 1 months, respectively. This is in contrast to the same virus in 4 ml volumes in ampules which showed no loss in titer after 29 months of storage at 4C. (ADP a8-20(R)

L. Survival and Transmission of Foot-and-Mouth Disease Virus in Semen.

Sixteen grade Hereford bulls were infected with foot-and-mouth disease virus (FMDV) by tongue inoculation. The virus strains used represented six of seven known types of FMDV. At various times after inoculation, semen was obtained from bulls by electroejaculation for inoculation into steers and suckling mice to recover virus and determine titers, and for insemination of heifers.

Foot-and-mouth disease virus was found in semen of 2 bulls as early as 12 hours postinoculation which was prior to appearance of clinical signs of infection. Thereafter, virus was found in semen of the 16 bulls in 55 of

65 attempts, for as long as 10 days postinoculation. The virus titer in semen was usually higher than in urine and sometimes higher than in blood samples taken simultaneously.

Artificial insemination techniques were used to place semen from infected bulls in cervical canal and vagina of 19 heifers. Four of the 19 heifers developed FMD. It was concluded that semen of bulls could contain FMDV prior to appearance of clinical signs and lesions of infection and that FMD may be transmitted by artificial insemination. (ADP a8-24)

M. <u>Identification</u>, <u>Purification</u>, <u>Characterization</u> of Foot-and-Mouth Disease <u>Virus</u>.

Foot-and-mouth disease virus was produced in roller bottle bovine kidney cultures. About 3 liters of virus were harvested each week containing 108.8 plaque-forming units per ml. Chemical and ultracentrifugal concentration and purification yielded 2 mg virus of at least 94% purity with maximal infectivities of 1011.0 PUF/ml., specific infectivities of 1014.0 PFU/gm and physical particles by electron microscope counting of 1013.6 virus particles/ml. The virus contained 32% ribonucleic acid (RNA) and 68% protein and had a 1% extinction coefficient at 259 mu of about 76. Its specific refractive increment was about 0.16 ml/gm. Viral RNA had an extinction coefficient at 258 mu of approximately 220 and a max258/min230 ratio of 2.1. The protein coat subunit of the virus obtained by heat, acid or urea treatments had a sedimentation coefficient (s20, w) of 12.2±0.3S. There is considerable, but yet unequivocal electron microscope evidence that FMDV is a icosahedron with 42 subunits in its coat protein. (ADP a8-25)

N. Immuno-Chemical Investigations of Foot-and-Mouth Disease.

Antibodies present in the blood serum of animals immunized against, or infected with, foot-and-mouth disease virus are known to play a major role in their resistance to subsequent exposure to the virus. It has been shown that these antibodies may be of two different types, and they appear at different times following initial exposure to either the living or inactive form of the virus. These two types of antibodies have different physical and chemical characteristics. The early appearing antibodies are larger molecules (19S sedimentation rate) than the later developed ones (7S sedimentation rate). They also migrate more rapidly under the influence of an electrical field (B-globulin mobility) than do the later ones (y-globulin mobility). Other differences in physical-chemical characteristics have also been found. In addition, differences in their serological activity have been found. The late appearing antibody is able to fix complement while the early antibody is not. The latter finding is important in that it imposes a limitation upon our diagnostic capabilities. Knowledge of the physical, chemical, and immunological nature of these antibodies is vital for an understanding of how animals resist infections.

Progress on the immunological characterization of the foot-and-mouth disease virus and antibodies produced by animals in response to the virus is dependent upon the development of precise measuring techniques. Quantitative immunological measuring methods have been applied to purified and crude virus preparations successfully. These procedures have certain advantages over many regular physical and chemical methods in that only minute amounts are required for the tests, the tests are usually easier to perform, and they may often be done on samples containing contaminating substances that interfere with other assay procedures. Further refinement and application of these procedures will increase our knowledge of the structure of foot-and-mouth disease virus. (ADP a8-26)

O. Survival and Inactivation of Foot-and-Mouth Disease Virus in Meat and Meat By-Products.

It has been established that the primary sites where foot-and-mouth disease virus may survive in carcass and in boned meat from infected animals are lymph nodes, hemal nodes, blood clots and bone marrow or bone fragments. A new and previously unreported site for virus survival is the joint fluids. Virus was found in joint fluids as early as 12 hours and for as long as 5 days after inoculation of cattle. The virus survived in joint fluids throughout usual treatments given carcass meat. During boning operation, virus in joint fluids could contaminate butcher's knives and be spread over surfaces of cut meat. The protection afforded the virus by the joint fluid would permit virus survival under some adverse conditions on surface of meat chunks. Thus, virus in joint fluids could be a hazard in imported meat. (ADP a8-28)

P. Biological Mechanisms of Natural Resistance and Susceptibility to Foot-and-Mouth Disease Virus.

Mice vary in susceptibility to infection with foot-and-mouth disease virus. Factors which affect the susceptibility or which might be related to the variation in response have been investigated. (1) Suspensions of kidney cells from 1- and 2-week old mice produced FMDV earlier and to higher titers than cells from older mice. While 1- and 2-week old mice are in the most susceptible age range, this difference in virus multiplication might be related to factors other than the susceptibility of cell donors. (2) Mother mice are most susceptible to FMDV during the first two weeks post partum and then gradually become resistant. To determine if this development of resistance was associated with weaning of their young and a consequent decrease in milk production, a group of mothers was given new litters of 5- to 7-day old mice at weekly intervals to the 4th week post partum. Challenge with FMDV demonstrated that such mice were slightly more susceptible than similar mothers with original litters but much less susceptible than 6-day post partum mothers. (3) Pregnant as well as mother mice are susceptible to FMDV. Experiments demonstrated that mother mice maintained the high degree of sensitivity to bovine serum which they developed before mating but became less sensitive after delivery of their young. (ADP a8-29)

Q. Studies on Foot-and-Mouth Disease Virus.

A PL 480 Grant was made to Instituto Biologico, Sao Paulo, Brazil, to conduct studies on foot-and-mouth disease virus. A laboratory has been established for production of tissue cultures to use as a media for propagating foot-and-mouth disease virus, and in the routine isolation of the virus from specimens received from the field.

A swine kidney cell line has been propagated for more than 18 months. The cells have remained fully sensitive to at least 3 types of FMDV encountered in Brazil. Work on attenuation of FMDV by tissue culture methods in this laboratory is continuing but no significant findings have thus far been reported. (S3-ADP-2)

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PARASITES AND PARASITIC DISEASES OF CATTLE Animal Disease and Parasite Research Division, ARS

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of \$400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA PROGRAM

The <u>Department</u> has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the designated locations.

The <u>Federal</u> scientific effort devoted to research in this area totals 21.5 professional man-years. This effort is divided among subheadings as follows:

Ecological Factors Influencing Nematode Development 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effects of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Acquisition and Effects of Roundworm Parasites of Cattle, as Influenced by Diet 1.5 at the Animal Disease and Parasite Research Division, Beltsville Parasitological Laboratory, Beltsville, Maryland.

<u>Artificial Propagation of Protozoan Parasites</u> 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Host-Parasite Relationships of Coccidia 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Ecology and Immunology of the Cattle Lungworm 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle 2.0 at the University of California, Davis, under a cooperative agreement with the USDA.

<u>Investigations of Trichomonad Parasites</u> 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan.

Host-Parasite Relationship of Intestinal Worms Cooperia spp. 2.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Factors Influencing Internal Parasitism of Grazing Cattle 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

<u>Winter Coccidiosis (Bloody Scours)</u> 1.0 at the Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Montana Agricultural Experiment Station, Bozeman.

Anaplasmosis of Cattle 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and through a memorandum of understanding and other agreements in cooperation with State Experiment Stations in California, Illinois, Louisiana, Nevada, the State Veterinarian of Tennessee, the USDA Entomology Research Station, Kerrville, Texas, and The Delta Branch Experiment Station, Stoneville, Mississippi.

Investigations on Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle, are under way through a PL L 80 Grant, at the School of Veterinary Faculty, Montevideo, Uruguay.

The Interrelationship of Diet and Parasitic Infection in the Production of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

The Histochemistry of Gastro-Intestinal Nematodes of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

<u>Parasites of Cattle - Stephanofilarial Species</u> 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, University Park, New Mexico.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Ecological Factors Influencing Gastro-intestinal Nematodes.

Investigations made at Experiment, Georgia, under the auspices of the Animal Disease and Parasite Research Division's (ADP) Regional Animal Disease Laboratory at Auburn, Alabama, showed a significantly greater number of larvae of <u>T. axei</u> was recovered from cultures made with feces when the host calf was on a hay diet than when it ate a grain (corn) ration. Grain added to feces containing eggs of <u>T. axei</u> and <u>T. colubriformis</u> completely prevented the development of larvae in cultures when compared to cultures without the grain additive. Undigested grain (corn) washed from the feces passed by a calf on a grain ration also inhibited the larval development when added to feces from a calf infected with <u>T. colubriformis</u>. Experiments have shown that a grain ration (corn) appears to have an inhibitory action on the development in the feces of the larvae of two species of cattle nematodes (<u>Trichostrongylus axei</u>, <u>T. colubriformis</u>). (Georgia)

Experiments at the ADP Regional Animal Disease Laboratory, Auburn, Alabama, have demonstrated that rabbits are infected by ingesting the third-stage larvae of the nematodes, Trichostrongylus calcaratus, T. affinis and Obeliscoides cuniculi. Rabbits do not become infected by placing larvae on the intact skin if precautions are taken to prevent ingestion of such larvae by the host. Trichostrongylus affinis has been found in the cottontail (Sylvilagus floridanus) at Auburn, Alabama. Preliminary studies indicate that this nematode inhabits the cecum and appendix and has a prepatent period of 10-11 days with maximum egg production between 11 and 14 days post-infection. In guinea pigs immunized to the ruminant parasite, Trichostrongylus colubriformis, the immune response apparently was directed against the parasitic third- and fourth-stage worms. On the other hand, parasitic third-stage larvae failed to stimulate any immunity in guinea pigs, fourth-stage larvae elicited a measurable degree of immunity, and fourth- and fifth-stage worms, combined, stimulated an almost total immunity. (Auburn, Alabama) (ADP bl-6)

B. Artificial Propagation of Protozoan Parasites.

Work was continued at the ADP Beltsville Parasitological Laboratory (BPL) on the artificial propagation of protozoan parasites for the purpose of determining essential metabolites. Studies to develop methods of producing, under artificial conditions, the blackheat parasite in large numbers have been hampered by the unavoidable appearance of yeasts in the cultures. Amphotericin-B, an antifungal antibiotic, was found to control yeasts in culture without damage to the parasites, provided cream from cow's milk was incorporated in the culture medium. Without cream, the parasites grew poorly, if at all. Cholesterol compounds which the blackhead parasite can use for food were without effect when used in lieu of cream. (ADP b1-22)

C. Ecology and Immunology of the Cattle Lungworm.

It was found at the Beltsville Parasitological Laboratory that double vaccination with irradiated larvae of the cattle lungworm can be effective in protecting calves from becoming appreciably infected with adults of this parasite subsequent to a massive exposure to vigorous infective larvae of the worm, such as occur on pastures. However, the procedure was not invariably effective under experimental conditions. Variability in its efficacy was not dependent on rate of irradiation of the vaccine within the limits that have been tested. Inherent individual variation among calves in capacity to develop resistance appears to be a significant determinant of the efficacy of vaccination.

Calves can become highly resistant or immune to the development of a mature cattle lungworm infection as a result of vaccination with infective larvae of a lungworm of sheep. The experiments on which this conclusion is based appear to be the first to show that one species of livestock can be immunized against one of its worm parasites by exposing it to infection with a worm that inhabits a different species. (ADP b1-24)

D. Clinical and Physiological Aspects of Roundworms in Cattle.

The School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, reported research to show: Phenothiazine prepared with an anhydrous aluminum chloride catalyst was found to be superior to phenothiazine N.F. and equal to purified phenothiazine when evaluated in mice infected with Nematospiroides dubius: The addition of the other insoluble impurities of phenothiazine N.F. to purified phenothiazine did not reduce the anthelmintic action of the latter product, and that thiabendazole was found to be highly efficient as an anthelmintic in clinical cases of gastrointestinal parasitism of cattle when used at a level of loo mg/kg body weight.

Work was also conducted on iron metabolism and hemopoiesis. The studies, utilizing Fe⁻⁵² were conducted in order to evaluate the red blood cell survival times in lambs as determined by the method of Baker and Douglas and that of Pollycove and Mortimer. It was found that the latter method gives a longer mean survival (131 days) than the former method (81.0 days). Definitive studies in 2 lambs, using the method of Eadie and Brown, indicated mean red cell survivals of 141 and 143 days.

Body surface monitoring of spleen, liver, and bone marrow indicated that the spleen of sheep may act as a labile storage pool for iron utilized in hemoglobin synthesis.

Analysis of tissues for Fe⁻⁵⁹ between 1.1 hr. and 7 days following intravenous injection of Fe⁻⁵⁹ labeled plasma in lambs, revealed that 4 to 21% of the injected isotope was in the liver, 0.00 to 2.5% in the spleen, 0.00 to 16.5% in muscle, 0.10 to 2.1% in the kidneys, and the remainder was in plasma, bone marrow, and red cells. (California) (ADP bl-25)

E. Investigations of Trichomonad Parasites.

Research on this project at the ADP Regional Animal Disease Laboratory, Logan, Utah, resulted in the following findings: Electrophoretic methods used for protein separation were not practical for immunoelectrophoresis because large amounts of antigen are required for the immunodiffusion phase of the procedure. The apparatus constructed for this method was therefore adapted to a micro-method. The supporting medium was changed from starch gel to ionagar which does not allow separation of as many fractions or as sharp delineation as starch, but the separation is sufficient for immunodiffusion. For general antigenic characterization and comparison of antigenic makeup of various strains of T. foetus, the micro-method should suffice. By this method rabbit anti-T. foetus serum was separated into the 5 fractions. The diffusion phase of the technique has not been combined with the electrophoretic phase, but has been done separately and produces precipitin lines. For positive identification of antigens that may characterize or differentiate strains, the starch gel method may be more effective.

Anti-serum against \underline{T} . <u>foetus</u> may be produced in rabbits by intravenous injections of washed live organisms. However, some rabbits do not tolerate the infection. Intracardial injections did not prove satisfactory, neither did the intraperitoneal method with any of the 4 strains of \underline{T} . <u>foetus</u> used. The effort to produce antiserum against \underline{T} . <u>foetus</u> in calves gave negative results.

Antigens for use with the gel diffusion phase of the immunoelectrophoretic technique to date have been the complete type consisting essentially of concentrated ruptured organisms. Three methods were used for rupturing the organisms—1) lysis by hypotonic solutions, 2) alternate freezing and thawing, and 3) blendor grinding. Acceptible antigens have been prepared from freeze-thawing. However, blendor-ground organisms gave most consistent results.

Gel diffusion reactions were produced in ionagar gel of several strengths prepared with several buffers of varying pHs. Therefore, it is anticipated that the gel diffusion procedure may be combined with the microelectrophoretic technique. In trials to date, however, considerable cross reaction between the two strains used have occurred. T. foetus was isolated from 10 of 280 samples taken from bulls during the year. This included herds under observation following known infection. (Logan, Utah)

Research was continued at the Utah Agricultural Experiment Station, Logan, under a cooperative agreement with the USDA, to learn more about pentatrichomonads that were isolated from the rumen and cecum of calves in northern Utah in 1961. This trichomonad grew in Diamond's modified Plastridge's, and cecal-extract media. No difference in response to different media or in morphology was found in the trichomonads from the rumen

and cecum. There were 3, 4, or 5 unequal anterior flagella, one of which was independent, a relatively high, full-length undulating membrane, a prominent costa, a narrow axostyle with long protruding tip and no chromatic ring, a subspherical or round-oval nucleus, and an oval parabasal body with one or more central granules. Mean measurements of 100 Bouin's-fixed, protargol-stained, rumen specimens from 24-hour Diamond's cultures were as follows: length, 7.8 microns (range, 5.7-10.0); width, 6.1 (4.2-7.9); protruding tip of axostyle, 4.0; anterior flagella, 9.7, 8.8, 7.2, 6.0, and 4.2; trailing flagellum, 5.4; nucleus, 2.5 by 2.0; parabasal body, 1.5 by 0.9; height of undulating membrane, 1.7. This trichomonad is similar to Pentatrichomonas hominis and may be identical with this species. (Utah) (ADP b1-26)

F. Host-Parasite Relationship of Intestinal Worms Cooperia spp.

Reported research from the ADP Regional Animal Laboratory, Auburn, Alabama, showed that calves inoculated with 250,000 Cooperia pectinata infective larvae made an average weight gain of only 6 pounds in 6 weeks, while non-inoculated controls averaged a gain of 25.3 pounds. Clinical signs of parasitism -- anorexia and enteritis as indicated by passage of abnormally soft stools -- appeared during the third and fourth weeks of infection, and these were accompanied by decreased levels of serum proteins and blood sugars. The effects were not as severe as those produced in previous studies wherein calves were inoculated with 350,000 infective larvae. Calves inoculated with 350,000 Cooperia oncophora larvae developed a mild parasitism, characterized by a short period of enteritis and some retardation on rate of gain. However, this parasite is not as pathogenic as a closely related species, C. pectinata. The intestinal worm, Cooperia pectinata, develops in the intervillar spaces of the duodenal mucosa, causing disruption of the intestinal villi, and evoking a catarrhal exudate.

It has been concluded from experiments at Auburn that some factor, or factors, operating within individual host animals (calves and lambs) are sufficient to affect significantly the size of infective third-stage larvae (Cooperia oncophora) developed from eggs laid by the infecting parasites. Some of these factors may be individual differences in the utilization of the diet by the host, or differences in the microbiota of the feces. (Auburn, Alabama) (ADP bl-27)

G. Factors Influencing Internal Parasitism of Grazing Cattle.

The Beltsville Parasitological Laboratory (BPL) research workers reported that calves and older cattle infected with nematodes were maintained on pasture in the summer and fall. During the first two months following infection, the rotated animals gained better than the non-rotated animals. This advantage was not maintained by the calves for the remaining two months of the experiments. In general, the worm burden of the rotated animals was as great as that of the non-rotated animals. The age of the animals had a greater effect on worm burden than rotation.

Mature cattle up to the age of at least 3 to 4 years are as susceptible to initial infection with the beef measles worm as are calves. The measles persist for more than 2 years. Consequently, sanitary measures for prevention of infection with this parasite, which causes losses by condemnation and special processing of carcasses, should be employed not only during calfhood, but also until the cattle go to market. (ADP bl-28)

H. Winter Coccidiosis (Bloody Scours) of Cattle.

Studies on this project were continued at the ADP Regional Animal Disease Laboratory at Logan, Utah. Washed sporulated oocysts of Eimeria bovis were injected either intraperitoneally, intravenously or subcutaneously into young calves in an attempt to establish an immune response. No reactions occurred in any of the calves as a result of the injections. No intestinal infections with coccidia occurred. Six weeks after these injections, oral inoculations with E. bovis oocysts were given to these calves plus a group of previously uninjected control calves. Coccidial infections developed in all four groups of calves indicating an absence of immunity in those previously injected with oocysts. Upon recovery from infection, the calves were divided into 3 groups. One group received an intramuscular injection of hormone (ACTH), and another group received an intramuscular injection of cortisone acetate. Later the 3 groups were given oral inoculations of E. bovis oocysts to challenge their immunity. There was no significant reinfection. Immunity apparently develops only as a result of intestinal infection. No changes in the blood serum potassium and sodium levels were detectable until shortly before death of the animals.

In studies to determine the number of oocysts required to produce an active immunity, 3 calves were given daily oral inoculations of 1,000, 5,000, and 15,000 $\underline{\text{Eimeria}}$ $\underline{\text{bovis}}$ sporulated oocysts, respectively, for 47 days. No significant difference was determined in the degree of immunity. Cross inoculation tests showed that severe infection with $\underline{\text{E}}$. $\underline{\text{bovis}}$ did not protect against infection with $\underline{\text{E}}$. $\underline{\text{zurnii}}$.

One of two calves inoculated with sporulated oocysts of <u>Eimeria zurnii</u>, refrigerated at 5°C for more than 2 years, died with severe symptoms. The other calf developed less severe symptoms and survived. Cortisone acetate injected subcutaneously in young calves was used in an attempt to develop a stress method for establishing consistent infections with <u>E. zurnii</u>. Oral inoculations of injected and uninjected control calves resulted in inconsistent infection. (Logan, Utah)

Research workers at the Montana Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, under a cooperative agreement with the USDA, determined from observations on 8 disease outbreaks in cattle tentatively identified as "winter" coccidiosis, indicated that Eimeria zurnii was the predominant organism occurring in 4 cases, E. bovis in 3, and E. canadensis E. brasiliensis each in 1 case. Final diagnosis of clinical coccidiosis was made in only 3 instances in which E. zurnii occurred alone. A severe

case of bloody diarrhea was observed in a mature cow in which the ciliate protozoan <u>Buxtonella</u> <u>sulcata</u> apparently was the causative agent. (Montana) (ADP b1-29)

I. Anaplasmosis of Cattle.

At the Beltsville Parasitological Laboratory, research workers reported the following findings: Ten trials to determine if females of the tick,

Dermacentor andersoni, can transmit Anaplasma marginale "hereditarily," or not, were conducted using splenectomized calves. All of these trials proved negative for this type of transmission of the anaplasm from adult to larval ticks.

Agar-gel double diffusion studies revealed that precipitating antibodies do occur naturally in the serums of cattle affected with anaplasmosis. Complement-fixing antigen served as the precipitating antigen in these studies.

Electron microscopy and immunofluorescent studies have indicated that all but one isolate of \underline{A} . $\underline{marginale}$, thus far studied, have appendages which cannot be seen with conventional microscopes. This appendage assists in the identification of the parasite in preparations made from infected ticks.

A commercial, chemically purified isomer of fluorescein isothiocyanate was found to yield optimum brilliance when conjugated to immune bovine serum, which was then used to stain blood films containing \underline{A} . $\underline{marginale}$.

The resistance of carriers of \underline{A} . $\underline{\text{marginale}}$ to re-exposure with the same agent was studied. It was found that some carriers withstood re-exposure, while others developed acute signs of infection.

Epizootiological studies over a 6-year period in a dairy herd in southern Louisiana have shown that acute anaplasmosis caused a 26 percent loss in milk production in lactating cows. There was no appreciable decrease in milk production of infected cows during the carrier stage.

A beef herd at the Kerrville Station, Kerrville, Texas, which in 1958 was heavily infected with anaplasmosis, has now become free of the infection. Clean heifers were used to replace their infected dams. During this year, the few animals remaining in the "reactor" herd were removed. Complement-fixation tests made at weaning time last August showed 7 reactors and 11 negative in 18 calves from the reactor cows. The 8 calves from the cows in the negative component were negative. During the February 19, 1963, complement-fixation testing, 60 days after vector peaking, one 2-year-old heifer was found to have a positive (1:10) titer. She was removed from the herd, kept in isolation and periodically retested. At the end of fiscal year 1963, she continues to give a "doubtful" reaction. At this time the

herd consists of 1 bull, 37 cows and heifers 2 to 4 years old, 9 yearling and 20 calves. A total of 383 blood samples were obtained and processed for complement-fixation testing. (Kerrville, Texas) (ADP b1-30)

Under a PL 480 grant to the School of Veterinary, Montevideo, Uruguay, research was conduted on anaplasmosis, piroplasmosis, and Babesialosis of cattle. The report of the work shows considerable progress has been made.

Bovine erythrocytes, some containing Anaplasma, and some containing Babesia, were washed and inoculated into cultures of both swine kidney cells and Hela cells. Growth of the parasites was not observed. Cellular deterioration occurred within 5 days. Hela cells were affected to a lesser degree than swine kidney cells. Cultures of splenic tissue of rats did not deteriorate following addition of erythrocytes from bovines in the clinical phase of anaplasmosis.

Electrophoretic studies were made of the serums of many experimentally anaplasmosis-infected bovines. Before anaplasms were detected in the peripheral blood, there was a decrease in the concentration of total serum proteins (TSP), of albumin, and in the albumin/globulin (AG) ratio, and a slight decrease in gamma globulin. The time when anaplasms were most numerous corresponded to the lowest level of TSP, albumin, gamma globulin, and of the albumin/globulin ratio.

Alpha and beta globulins increased during the entire course of the clinical disease, reaching their highest levels at end, 43 days, and then diminishing to pre-infection levels. The concentration of gamma globulin increased as the level of the alpha and beta globulins returned to normal. The concentration of gamma globulin was highest at the end of the study, 65 days after infection. The concentration of antibodies appears to be greater in the alpha and beta globulins than in the gamma fraction. Changes were not observed in control animals.

Anaplasmosis-experimentally-infected bovines were inoculated, intravenously, with 2 doses of Spirotripan, 20 cc per dose. Some animals were inoculated during the time the anaplasms in the blood were on the increase; others were treated during the height of the infection. These treatments were ineffectual in altering materially the natural course of the infection or of the disease.

The "mechanism of anemia" in anaplasmosis was studied in splenectomized rats, experimentally infected with <u>Anaplasma ratti</u>. A transient anemia, of a few days duration, was observed. Intracellular bodies in erythrocytes of 2 animals were observed. The bodies bore some resemblance to anaplasms, but could not be positively identified.

Tissue culture techniques were used to produce ovarian, muscular, and "glandular" cells of the tick <u>Boophilus microplus</u> for use as a media in the study of developmental stages of <u>Babesia</u> and <u>Anaplasma</u>. Only "a survival

phenomenon" occurred and, in some cultures of ovarian tissues, limited development of the ovocites. (Uruguay) (S9-ADP-1)

J. Parasites of Cattle - Stephanofilarial Species.

This is a preliminary report on a new project initiated at the ADP Regional Animal Disease Laboratory, University Park, New Mexico. It concerns studies of worm parasites of cattle on irrigated pastures and on high-rainfall areas of the Southwest, with special emphasis on the Stephanofilarial species. Stephanofilaria stilesi has been found in 26 of 28 (93%) beef cattle examined. The lesions caused by this nematode were usually restricted to the region of the brisket in young animals and were from 1 to 2 inches in diameter, but in older cattle the lesions often extended from the brisket to the udder or scrotum, involving as much as 2 square feet of skin. Ten of 25 (40%) dairy animals examined also had lesions typical of the disease. As in beef cattle, the udder was often involved. A study of the mode of transmission of the parasite is in progress. (New Mexico) (ADP bl-33)

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BEEF CATTLE, HORSE, AND SWINE INSECTS Entomology Research Division, ARS

Insects and ticks irritate and torment livestock throughout the year in all parts of the United States and cause serious losses. These pests reduce weight gains, lower the quality of meat and hides, and spread numerous animal diseases. Livestock losses directly attributable to insects and ticks are estimated to exceed \$300 million annually. Practical but not adequate control methods have been developed for lice, screw-worms, ticks, bots, grubs, and other insects, but satisfactory methods of protecting cattle from horse flies, deer flies, stable flies, mosquitoes, and the newly introduced face fly remain an unsolved problem. The development of insecticides for use on beef cattle, horses, and swine has been hampered because certain insects have become resistant to various insecticides, and because harmful residues have been found in meat following the application of certain materials. Safe, effective, nonresidue-forming insecticides and repellents are required. Urgently needed are economical and long-lasting insecticides or repellents for range cattle to protect them against vicious biting flies. Safer, cheaper, and more effective systemic insecticides and more efficient means of administration are needed to combat grubs and bots in cattle and horses. New approaches to control, including radiation and chemosterilants, should be explored to determine their feasibility as practical control methods. Efforts also need to be made to find and evaluate insect pathogens, parasites, and predators for controlling certain livestock pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycles that will serve as a basis for the development of more effective and safer methods of control. Research also is urgently needed on the role of insects in the spread of diseases of livestock.

USDA PROGRAM

The Department has a continuing, long-term program involving basic and applied research on insects and ticks which affect the health and productivity of beef cattle, horses, and swine. Studies are conducted on the biology, physiology, genetics, and nutrition of the screw-worm, stable fly, horn fly, house fly, mosquitoes and other pests; on the nature of insect resistance to insecticides; and on absorption, metabolism and excretion of insecticides by insects feeding on or in animals; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is concerned with the development of more effective contact and systemic insecticides and protective treatments for the control of livestock pests. Studies are conducted to determine the occurrence of residues in tissues of animals treated with insecticides. Minor consideration is given to the development of sanitation and management procedures and biological control methods, including parasites and predators, for controlling the face fly, stable fly and several other pests. Emphasis is also given to the development of insect sterility, attractants and

various other noninsecticidal approaches to control. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to evaluate various kinds of traps and devices for estimating and controlling natural insect populations, and improved or special equipment for the application of insecticides to animals. Limited research is conducted on the role of insects and ticks as vectors of livestock diseases, with particular emphasis on bovine anaplasmosis and equine piroplasmosis. The Federal scientific effort devoted to research in this area totals 16.3 professional man-years. Of this number, 6.1 is devoted to basic biology, physiology and nutrition; 3.9 to insecticidal and sanitation control; 2.0 to insecticide residue determinations; 0.2 to biological control; 2.0 to insect sterility, attractants and other new approaches to control; 0.2 to the evaluation of equipment; 0.8 to insect vectors of diseases; and 1.1 to program leadership.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology and Nutrition

1. Mosquitoes. At Corvallis, Oreg., and Fresno, Calif., studies were continued on the biology and ecology of important mosquito species. In laboratory tests the majority of <u>Culex tarsalis</u> females preferred to feed at the higher of two feeding locations and preferred mice rather than chickens for their source of blood.

A study was conducted on the daily activity of several species of mosquitoes ——Anopheles freeborni, Culex peus, Culiseta inornata, Culex incidens, and Culex tarsalis. All species behaved similarly. Both males and females began to move out of daytime resting shelters a few minutes before or after sunset. Only a few males and females (0.3 to 2.8%) remained in the shelters during the night. Adults did not begin to return to the shelters until sunrise or a few minutes later, and all did not return until sometime after 8:00 a.m. Exodus from and return to shelters appeared to be regulated by light intensity. Other factors such as preconditioning to light cycles, temperature, and humidity, may have had some effect since artificial lights did not prevent mosquitoes from leaving shelters eventually, but did change their time and rate of leaving. Use of special crosses of resistant and susceptible strains of Culex tarsalis showed that some of the females mate more than one time and utilize sperm from more than one mating.

Taxonomic studies defined suitable characters for separating dark-winged forms of Aedes dorsalis from A. melanimon and determined that these two forms coexist in only one area (Solano County, Calif.). Surveys at Borrego Springs (San Diego County, Calif.) were made to find isolated populations of mosquitoes for future studies on control through sterilization.

Surveys were continued on biting arthropods of the Humboldt River Basin in Nevada. During April, May, and June, water was plentiful and high populations of Aedes melanimon, A. dorsalis, A. vexans, Culex tarsalis, and Culiseta inornata were found. Mosquito larvae were found infected with a

microsporidian, Thelohania sp. and a bacterium, both of which are under study as biological control agents. In late July, no floodwater Aedes breeding was observed, but moderate breeding of Culex tarsalis and Culiseta inornata was observed. Studies of mosquito larval populations in typical breeding areas in dairy drains and culverts in California during April indicated the presence of Culiseta inornata, Culiseta incidens, Culex tarsalis, Culex peus, Culex apicalis, and Anopheles franciscanus. No breeding of Culex quinquefasciatus was found.

In Oregon, using C-l4 labeled TDE, slight but consistent differences in the rate of degradation of TDE have been noted in susceptible and resistant strains of <u>Culex tarsalis</u>, indicating that the main source of resistance to this chemical is increased degradation of TDE to FW-152 and water soluble metabolites through an oxidative rather than a dehydrochlorination mechanism. Further tests with DDT and TDE have shown that analogs of DDT, which block metabolism via the oxidative pathway, overcome resistance to DDT and tend to confirm the hypothesis that resistance to DDT and TDE in <u>tarsalis</u> is due to a more rapid oxidative metabolism of the insecticide.

Early in July 1962, reports were received of a serious mosquito outbreak in the cattle-growing areas along the Gulf Coast of Louisiana and Texas. Surveys in these areas revealed a heavy mosquito population resulting from successive broods of mosquitoes over a period of 6-8 weeks. At the time of the survey, the spring calf crop had been decimated and hundreds of mature cattle had perished from the attacks of mosquitoes. Growers had sold or moved thousands of animals to avoid further losses. The loss incurred by cattlemen was estimated at \$250,000. Because of the large area involved, control of the mosquitoes was not feasible.

2. Stable Fly. In Texas stable flies began to feed when they were only 4 hours old. The adults did not feed well or survive long on deer blood or several synthetic diets. They thrived on beef blood and beef serum but not on blood cells alone. At 80°F. and 50-60% relative humidity first matings occurred when the flies were 3 days old and 84% mated within 5 days. At 80°F. and 10-30% relative humidity mating did not begin until flies were 4 days old and only 12% mated in 5 days. No mating whatever occurred at 68°F. and uncontrolled humidity.

Stable fly larvae were not adversely affected by 17 hours of submergence in water, but 100% mortality resulted from 24 hours submergence. Exposure of pupae in water for 17 hours resulted in retarded development and greatly reduced adult emergence.

In Oregon field studies determined the dispersal pattern of marked adult stable flies. Flies were recovered over 14 miles from the point of release in 24-48 hours.

3. Face Fly. In Nebraska, face flies were first observed on cattle in the field on April 25 and by mid May they were present in annoying numbers. By early June heavy populations of 20 or more per head were widespread.

Studies on the behavior of the face fly were conducted in a large outdoor screened cage in which a calf was restrained. Several hundred laboratory-reared flies were released in the cage before each observation period. Less than 4% of the flies were present on the calf at any one time. The remainder rested in the grass or on the sides of the cage. Only an occasional male was observed on the calf. Nearly half of the flies congregated around the eyes and about 25% on the muzzle.

4. Horn Fly. In Texas, after considerable trial and error, a technique was developed for colonizing the horn fly in the laboratory without access to a bovine host. The diet consisted of 1 part bovine blood and 1 part beef juice plus 1 mg. of streptomycin, 1000 units of penicillin and 250 units of mycostatin per ml. of diet. Flies were fed by placing a cotton pad soaked with the diet on top of the holding cages. Cages consisted of 4" lengths of 6" plastic tubing with ends of plastic screen. Eggs were deposited on the plastic screen. Optimum conditions for adult reproduction were 90°F. temperature and 60-80% relative humidity. The colonization procedure is easy and inexpensive and greatly facilitates laboratory testing of insecticides, chemosterilants and repellents at all seasons of the year. Adult flies survived for 96 hours in a room kept at 75-80°F. and 62-67% relative humidity. Only a few survived in a room kept at 85-92°F. and 34-40% relative humidity.

In Oregon field studies determined the pattern of dispersal of marked adult horn flies. Flies were recovered over 7 miles from the point of release in 24-48 hours.

5. Screw-worm. Research on the screw-worm was discontinued at Kerrville, Tex., in September 1962 and moved to Mission, Tex., headquarters of the Southwest eradication campaign. This included research on the biology and ecology of the screw-worm under field conditions, vigor and longevity of sterilized flies, effects of radiation, the development of genetically marked strains, cytological studies with irradiated flies, nutrition, and special tests related to problems in the sterile male release program.

A new technique to determine the mating aggressiveness of sterilized or mutant screw-worm flies was developed. It was observed that in laboratory cages males harassed females sufficiently to cause mortality greater than that occurring when females were caged without males—in general, higher ratios of males to females caused greater mortality than lower ratios. Tests with various strains of screw-worms of known sexual competetiveness in comparison with other mating aggressiveness tests confirmed the validity of the method. Since the criterion for mating aggressiveness is the mortality of females over time rather than egg production and viability, the time, space, and labor required to determine sexual aggressiveness were

greatly reduced. Studies confirmed optimum numbers of screw-worm larvae per tray for rearing efficiency and optimum numbers of adults per cage for mating and longevity studies. They also confirmed diurnal periodicity in screw-worm pupae for emergence occurred from sunrise until noon. Studies indicated that the use of $\rm CO_2$ as anesthesia for screw-worms is safe for handling young screw-worm adults for longevity and mating tests and scanning for genetic markers.

Research was continued to find and develop genetically distinct strains of screw-worm flies and to study these mutant flies. In the adults many genetic markers were found such as yellow eyes and white auxillary region ("Whaxy"). The genetic marker "Whaxy" affects the morphology of both the adult and larvae -- a factor which would be invaluable in field studies. Approximately 270,000 screw-worm larvae were examined for morphological variants and from this 221 cultures were studied as possible genetic strains. Of markers found in the immature forms, at present three morphological characters -- interrupted bands of segmental spines, two spiracular openings in the anal plates of third-instar larvae, and additional spines on the eleventh anterior segmental band--were demonstrated to be genetic in nature. None of these were established as a pure strain. Research with these and other mutant strains is being pursued to determine the genetics of the strains and if these strains are as vigorous and aggressive as the normal strain, factors which are essential if the strains are to be used in the release program. For example, preliminary genetic studies indicate that the "Whaxy" mutant is lethal in the homozygous state with only a few individuals escaping. Other strains have slight behavioral differences from the normal strain, i. e., they respond differently to attractants and survey traps.

When pupae of the screw-worm were irradiated in an atmosphere of CO₂ and air (50-50 mixture), damage to the reproductive system of the adult female was greater than that induced by a similar radiation treatment in air. When the pupae were pretreated in the gas mixture for approximately 45 minutes, complete sterility was induced by a treatment of 4,500 r delivered in CO₂ and air, whereas irradiation in air alone required about 5,500 to 6,200 r. Techniques were developed to study the cytological effects of radiation and chemosterilants on screw-worm, particularly on spermatogenesis and oogenesis. Preliminary studies revealed chromosomal aberrations severe enough to cause dominant lethality. Considerable data on oogenesis and spermatogenesis in normal screw-worm flies were accumulated as necessary background data for evaluating the effects of radiation and chemosterilants on reproduction.

For rearing larvae of the screw-worm, a synthetic diet was developed that contained casein, yeast extract, cholesterol, inorganic salts, water, and agar. This medium was further defined by replacement of casein with a mixture of L-isomer amino acids, and of yeast extract with a mixture of B-vitamin and RNA. Larval growth and development on the defined medium were nearly equal to that on media containing casein and yeast. Larvae

absolutely required thiamine, riboflavin, panthothenate, niacin, and choline for growth. Biotin and folic acid stimulated growth and were necessary for maturation to the adult stage. Pyridoxine and its analogs, pyridoxal and pyridoxamine, inositol, B₁₂, and carnitine had no effect on growth. Niacinamide spared niacin, but p- aminobenzoic acid had no effect on the folic acid requirement.

Studies on the effect of dessication, lack of adult food, reduced temperature, age of flies at time of release, and the effect of all of these factors on different strains of the screw-worm were undertaken to improve the survival of screw-worm flies released into the hot and dry climate of Texas. Provision of food in release cartons or the judicious use of reduced holding temperatures curtailed mortality prior to or shortly after release. Techniques need to be developed which would allow greater uniformity in age of flies at the time of release. Newly emerged flies carry a food and water reserve that will sustain them up to 24 hours (90°F. and 30% R.H.) compared to only 3 to 4 hours for 2-day-old flies. Selection of flies for individuals more capable of survival under unfavorable conditions has shown promising results.

Extensive studies were made of the ecology of screw-worm flies under field conditions by releasing tagged flies. It was determined that flies tend to congregate and disperse along water courses or streams and are capable of traveling long distances. This research resulted in the addition of stretegic releases of screw-worms along water courses and an increase in the effectiveness of the sterile-male release program.

6. House flies. At Orlando, Fla., research on control or eradication through the use of sterilization by radiation or chemicals has shown that considerable gaps exist in knowledge of the biology and mating behavior of house flies. Mating tests have shown that both males and females must undergo a sexual maturation time of at least 16 hours with males and a minimum of 24 hours with females. Once the mating drive has started, males will attempt to "strike" or mate with both males and females and certain inanimate objects, although they "strike" more readily and frequently with females. This fact along with experiments in a large cage-type chemotactometer suggested the presence of some type of a female sex attractant of a low order. Imperfect sex recognition in the male combined with a low order sex attractant in the female would account for the fact that males attempt to mate with either sex and result in the higher ratio of male-to-female "strikes." Quantitative data on the male and female mating behavior and a detailed description of the mating "strike" of the male and mating behavior of the male and female have been developed. Males or females which had their wings removed were able to mate with individuals of the opposite sex with wings. Amputation of more than one pair of legs from the male prevented mating, while amputation of only one pair of legs impeded but did not prevent mating.

Tests were run to determine the actual time house flies remain in copula after initial seizure. Of 61 mating pairs that were trapped and observed, the shortest mating period was 44 minutes and longest, 96 minutes. The average was 60 minutes. Females examined after only 1 and 2 minutes of mating contained no sperm. Those examined after 3 to 5 minutes of mating had spermathecae which contained a few sperm and some which were completely filled. After mating for periods of 10 to 76 minutes, the spermathecae were filled to capacity with sperm, with only one exception.

Eighty-six female house flies reared in the laboratory laid an average of 94.4 eggs per female of which 89% hatched. The number of eggs laid varied from 12 to 186 per female. Under laboratory conditions some flies fed within an hour after emergence; others did not feed until later and some did not feed until they were 16 to 18 hours old.

At Corvallis, Oreg., studies were continued on the physiology of resistant and susceptible house flies. Isolan-resistant house flies have decreased ali-esterase activity and increased Isolan-detoxifying enzyme. Techniques using high-speed centrifugation have been developed which concentrate enzymes important in metabolizing or conferring resistance to organophosphorus compounds. In the case of malathion-resistant house flies, the nature of the alkyl groups attached directly to the phosphorus atom is the major factor in resistance. Thus, the primary cause of resistance is probably associated with the rate of recovery of the phosphorylated detoxifying enzyme after poisoning, rather than with an increased ability to cleave the toxic molecule per se.

In Oregon extensive studies were conducted on the biology, nutrition, and colonization of the little house fly (Fannia canicularis). After much trial and error a satisfactory rearing medium was developed which consisted of alfalfa meal, yeast extract solution and wood shavings. Adult flies were held in standard cages and provided with wrinkled balls of black muslin impregnated with alfalfa and yeast extracts for oviposition sites. Under such conditions 48% of the females and 25% of the males survived for 22 days in cages kept at 70-80°F. At 90°F. no males and only 8% of the females survived for 22 days. Egg deposition was about 5 times as great at 80°F. as at 70° or 90°F. Females began ovipositing in 5-6 days at 90°F., 8-9 days at 80°F. and 10-11 days at 70°F. The eggs dessicate rapidly and must be kept constantly on a moist surface to insure a high percentage of hatch.

7. Cattle Grubs. Research was continued in Texas and Oregon to determine the nutrition requirements of cattle grub larvae and to develop an artificial medium for rearing larvae under laboratory conditions. In Texas various methods and media were tried in an effort to rear grub larvae. First stage larvae survived as long as 13-15 days in several tests but no second and third instars survived more than a week.

In Oregon, studies were conducted to determine the optimum conditions for larval pupation and development. Pupae held in moist sand at 40°, 44° and 50°F. produced no adults. Some emergence occurred at constant temperatures of 60° and 68°F. and at fluctuating temperatures ranging from 63°-75°F. Emergence was greater under fluctuating temperatures than at constant temperatures. Examination of pupae indicated that failure of adults to emerge was due to inability to eclose.

- 8. Horse Flies and Deer Flies. Studies were continued in Mississippi on the biology of tabanids. First adults of Hybomitra lasiophthalmus were noted on March 28. By mid-April this species was abundant and small numbers of T. vittiger schwardti and T. fusciacostatus were present. The usual succession of species appeared during the summer and early fall months but none reached their usual abundance. Population declined drastically during the latter part of July as a result of prolonged drouth. Populations rose only slightly following rains in late August.
- 9. Lice. Studies in Oregon showed that low louse populations on cattle during the summer months are largely due to destruction from licking. Short summer hair coats do not protect the lice, whereas long winter coats do. When short coated animals were restrained from licking themselves, heavy louse populations developed in 3-4 weeks.
- 10. <u>Ticks</u>. Observations in Texas showed that the lone star tick became active in January 1962, and population on cattle increased gradually during February, March and April, reaching a peak in May and June. Tick populations began to decline in July and had virtually disappeared by September.

The winter tick first appeared on cattle in October 1962, and populations gradually increased to a peak in December 1962, and January 1963. Tick populations declined rapidly in February and had virtually disappeared by late March.

B. Insecticidal and Sanitation Control

1. Mosquitoes. Studies were continued at Orlando, Fla., to find new and effective insecticides for the control of mosquitoes. In screening tests with Anopheles quadrimaculatus larvae, 28 of 81 compounds tested were rated Class IV in effectiveness. Four of these compounds—American Cyanamid CL-18133, Stauffer B-10046, Stauffer 8-10094, and Bayer 47940—were highly effective, killing 100% of the larvae at concentrations of 0.05 to 0.01 p.p.m. One hundred and nine plant extracts were also screened for toxicity, but none of these were toxic at low concentrations. Of 83 compounds tested against female Aedes taeniorhynchus in wind tunnel tests, 7 were as effective as the standard, malathion. Dimethrin in granular formulations was effective in laboratory and field tests as a larvicide against Anopheles quadrimaculatus, Aedes aegypti, Aedes taeniorhynchus and Culex quinquefasciatus mosquitoes. A mixture of DDT and an anti-resistant compound was no more effective than DDT alone against C. quinquefasciatus larvae.

In Oregon, studies were continued to find repellents for protecting animals from attacks by mosquitoes. Only one (ENT-26455) of 91 compounds tested was a highly effective repellent. This material showed 100% repellency for 24 hours and from 50 to 90% repellency after 48 hours. Several materials were highly effective toxicants but none was superior to the standard, Bayer 29493.

In Oregon, 46 compounds were tested for systemic action by giving them orally to mice and allowing mosquitoes to feed on the mice. At a dosage of 100 mg./kg., two compounds--Bayer 29493 and Shell SD 8436--killed all mosquitoes for 6 hours after the mice had been treated.

2. Stable Fly. In Texas, 240 compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Of these materials, 6 were Class IV repellents at 5% and 21 were Class IV toxicants at 0.5%. A number of other materials were Class IV at higher concentrations. The outstanding repellents were ENT-20274, ENT-25927, ENT-26864, ENT-32965, ENT-25946, and ENT-27031.

Tests were conducted with the WHO test kit to determine the susceptibility of non-resistant stable flies to DDT and dieldrin. At an exposure of 30 minutes the LD-50s and LD-90s for DDT were 1.7 and 2.6% and for dieldrin 0.36 and 0.91%, respectively.

In field tests in Nebraska, applications of 1 pint of 0.5% Ciodrin to cattle gave good control of stable flies for 3-4 days. Daily applications with an automatic sprayer of 100 cc of 0.8% Ciodrin maintained very good control of stable flies. In field tests in Texas, sprays of 0.25% DOWCO 175 (ENT-25964) applied at the rate of 2 quarts per animal gave excellent control of stable flies for 3 days.

3. Face Fly. In Nebraska, tests were conducted in which materials were administered to cattle in feed in order to determine their effectiveness in preventing fly breeding in feces. Concentrations of 0.1% and higher of Bacillus thuringensis, an insect pathogen, prevented fly larval breeding in droppings. At concentrations of less than 0.1% larval survival increased in proportion to the decrease in concentration. Phenothiazine (2.5-3 microns) administered at the rate of 2 grams daily for 12 days gave erratic results but in no instance provided effective control of larval breeding. A concentration of 20 p.p.m. of Co-ral in feed completely inhibited larval breeding and DDVP at 0.5 mg./kg. was almost completely effective. Free choice consumption of salt containing 0.5 or 1.0% Co-ral gave erratic results, larval control ranging from 40 to 85%. The erratic results were attributed to differences in consumption (0.06 to 0.35 mg./kg. daily) by individual animals.

In Nebraska, tests were conducted in which 4% malathion and 0.8% pyrethrins plus 1.1% piperonyl butoxide were installed around salt boxes. Daily contact with the dust bags caused reduction in face fly populations on cattle.

4. Horn Fly. Extensive field tests were conducted in Texas, Mississippi, Nebraska, and Oregon, to compare several old and promising new insecticides for the control of horn flies on cattle. In Texas sprays of 0.25 and 0.5% Hooker 1422 (ENT-25780) applied at the rate of 2 quarts per animal provided effective control of horn flies for 7-10 days. Applications of 2 quarts of 0.5% Ciodrin and 1 pint of 2% Ciodrin provided 14-95 days control in dry central Texas and 11-12 days control in humid east Texas. Tests were conducted with several materials applied in minimal amounts by an automatic mist sprayer. Daily applications of 0.2% or 0.5% of DDVP provided excellent immediate control and kept animals entirely free of horn flies. Spraying on alternate days kept populations at a sub-annoying level. Similar results were obtained by spraying daily with 0.125-0.25% Ciodrin. Single applications of 0.5, 0.75, and 1.0% Ciodrin provided effective control for 10, 14, and 14 days, respectively.

In Mississippi, sprays of 0.5 or 1.0% malathion applied at the rate of one-half pint per head provided excellent control for 4 days and satisfactory control for about a week. Similar applications of 0.5% Cygon, methoxychlor, toxaphene and Famophos were effective for 3, 4-5, 4-5, and 5-6 days, respectively. Applications of 2 quarts per animal of 0.5% toxaphene or Co-ral provided effective control for 3-5 and 11 days, respectively. In tests with automatic sprayers, applications of 6 ounces (3 round trips per animal) in one day of 0.1% Baytex, 0.15% Delnav, 0.5% malathion, 0.5% toxaphene, 0.5% Bayer 22408, 1.0% Ciodrin, and 1.0% Ciodrin plus 0.25% DDVP provided effective control for 5-6 days. Ronnel and Baytex at 0.5% were effective 3-4 days. Similar applications of 0.1% Baytex, 0.5% Cygon and 0.5% Bayer 22408 were effective 1-2 days.

In Nebraska, 2 quart applications of 0.1 and 0.25% Stauffer R-1504 (ENT-25705) gave good control of horn flies for 11 days.

Tests were conducted to determine the effectiveness of low-level feeding of a number of materials in preventing horn fly breeding in cattle droppings. In Texas, daily feeding of Bayer 29493 at 1 mg./kg., Bayer 37341 at 2.5 mg./kg. and Bayer 37342 at 5 mg./kg. gave 100% control of larval breeding. Three commercial brands of Bacillus thuringensis administered at concentrations of 0.05-0.1% in feed also prevented fly breeding in droppings. These materials gave similar results in free choice feeding tests but the effective concentration of two of the materials was about 4 times that of the third one.

In field tests in Oregon, one-quart applications of 1% Shell SD-8436 (ENT-25840) gave highly effective control for over a week and 0.25% was moderately effective for this period. Similar applications of 0.25% Ciodrin, Bayer 29493, and Famophos, and 0.5% malathion, were effective for 4 days. Five insecticides were evaluated at high concentrations applied at low volumes (12 ml) to the backs of cattle with a pump oil can. In these tests 4% DDT was effective more than 21 days, 4% methoxyclor for 11-15 days, 4% malathion for 7-9 days, 4% Barthrin for 7 days and 5% Barthrin

for 14 days. In feeding tests, single oral administrations of 50 mg./kg. of ENT-25842 inhibited fly breeding in feces for 6-9 days. A similar dose of ENT-25840 was effective 4-5 days. A dose of only 1 mg./kg. of either compound was effective for 1 day. In tests involving feeding over a period of 5 days Zytron at 5 mg./kg. or carbophenothion at 10 mg./kg., prevented breeding in feces the first day. Lower doses of these materials and Bayer 29493 inhibited breeding after 3 days. Famophos 20 mg./kg. and Stauffer R1504 at 10 mg./kg. were not completely effective.

6. House Flies. Research was continued at Orlando, Fla., to develop more effective insecticides and other methods and materials for the control of house flies. Twenty-three new compounds were tested as space sprays in a wind tunnel against the regular susceptible colony and the Cradson (multiresistant) colony. Fourteen of the compounds were more effective against both the susceptible and resistant colonies than the standard, malathion. New insecticides were also evaluated as residual treatments against female house flies from the regular or Cradson colonies. criterion of effectiveness was the number of weeks of aging during which the residues remained effective in killing house fly females. Against susceptible house flies Hercules AC-5727 alone and Bayer 39007 alone gave kills of 90% for one week, but in combination with Monsanto CP-16226, their effective periods were extended to 8 weeks and 12 weeks, respectively. The effectiveness of Hercules 7522H was also extended from 1 to 12 weeks with the addition of Monsanto CP-16226. Against flies of the Cradson colony all of these formulations failed before the fourth week. Against the regular colony Bayer 29952 and Bayer 30237 were effective for 64 and 56 weeks. Stauffer N-2230 and Stauffer N-2404 were 100% effective throughout 48 weeks of aging against the susceptible colony, but they were ineffective against the Cradson colony. General Chemical GC-3583 was still 100% effective after 96 weeks against the regular colony and Monsanto CP-40294 was effective for 24 weeks against the Cradson colony. Against house flies from the susceptible colony, General Chemical GC-4072 was 100% effective for 96 weeks as an acetone solution and Stauffer N-2310, Bayer 39197 and Monsanto CP-40273 for 48+ weeks. As wettable powder DDT was more than 90% effective for 48 weeks, Bayer 25141 for 48 weeks, Bayer 34098 for 40 weeks, Hooker HRS-1422 for 32 weeks, and Bayer 32651 for 28 weeks.

Residual tests (deposits of 100 mg./sq. ft.) were conducted with emulsions of diazinon, Baytex, and dimethoate against house flies in Florida dairy barns. The diazinon treatment failed to give satisfactory control as early as the first day after treatment. Baytex gave 97% control of 5 days and from 79 to 88% control through 14 days. Dimethoate gave controls ranging from 80 to 96% for 6 weeks, when the test was discontinued.

At Corvallis, Oreg., extensive studies were continued on the development of synergists that have been shown to overcome resistance to organophosphorus insecticides in both house flies and mosquitoes. Of some new types of compounds screened, results indicate that disopropyl or dibutyl

substitutes would be most satisfactory. Selection of house flies with combinations of malathion and synergists are being carried out to determine if resistance to the combinations can be developed.

7. Cattle Grubs and Other Bots. Research was continued in Texas and Oregon to develop more effective insecticides for controlling cattle grubs and other bots affecting livestock. In Texas 79 new compounds were screened for systemic action by giving them orally (0), subcutaneously (SC) or intramuscularly (IM) at several dosages to guinea pigs infested with larvae of the screw-worm, Cochliomyia macellaria or Phormia regina. Nine of the materials showed systemic action in one or more types of administration. The active compounds and minimum effective dosages in mg./kg. were as follows: Stauffer R10094, 15 mg. 0 and 25 mg. SC; Shell SD-8448, 50 mg. SC; Shell SD 8530, 100 mg. 0 and 50 mg. SC; Chemagro S-8550, 100 mg. 0; Hercules 7845, 10 mg. 0 and 25 mg. SC; Stauffer R-6032, 25 mg. 0; Monsanto CP 19203, 10 mg. 0 and SC; Shell SD 8280, 10 mg. SC; and Pyramat, 100 mg.SC.

In Texas tests were conducted on small numbers of animals (2-4) with a number of compounds that had shown promise in screening tests or on individual cattle in 1961-1962 and with older effective materials administered in different ways. The effective materials, dosage, and route of administration were as follows: Co-ral, 2 and 8% pour-on; Bayer 37341, 2% pour-on; Bayer 37342, 2% pour-on, and 15 mg./kg. IM; Famophos, 20 mg./kg. IM; Stauffer R1504, 2% pour-on and 2.5 mg./kg. in feed for 10 days; Rhodia, 15 mg./kg. in feed for 10 days; Dipterex and Butonate, 5 mg./kg. in feed for 10 days; and Baytex, 2.5 mg./kg. in feed for 10 days. All of these treatments gave 97-100% control of the two species of grubs which infested the Wyoming cattle used in these tests.

Extensive field tests with Government and cooperator herds of cattle were conducted in Texas and Oregon to evaluate the effectiveness of promising new and several older systemics at different rates and various methods of administration. In Texas, a total of 30 materials were tested but only 7 produced 89-100% control. The effective materials, dosage and methods of application are as follows: Baytex, 1 mg./kg. in feed for 10 days; Dipterex, Butonate, and Bayer 37341, 2.5 mg. in feed for 10 days; Bayer 37342, 5 mg. in feed for 10 days; Famophos, 12.5% pour-on (65-125 mg. per animal); Imidan, 2% pour-on (125 mg.) and 10 mg./kg. IM.

In Oregon extensive tests were conducted with eight known systemics with the primary objective of determining the minimum effective dosage by pour-on and spraying. In pour-on tests, applications of 82 and 120 ml. of 12.5% Famophos in oil or glycol gave 99-100% control of grubs. Lesser amounts of these concentrations were slightly less effective (91-92% control). Oil applications of Famophos were slightly more effective than emulsions. Pour on applications of 60 and 120 ml. of 12.5% Bayer 37342 gave 95 and 96% control. With Bayer 29493 as little as 20 ml. of 10% in oil and 40 mg. of 5% in oil gave 92% control of grubs. Pour-on applications of 5 grams of Shell SD-8436 and 20 grams of Shell SD-8448 gave 100% control of grubs.

Oral administrations of 50 mg./kg. of both compounds failed to give satisfactory control.

In a series of spray tests, one gallon applications of 1, 1.5 and 2% Dipterex gave 97-100% control of grubs. Lower concentrations did not give satisfactory control. One-half % Ruelene sprays produced 93% control but at 0.2% control was only 80%. Sprays of Co-ral at 0.5% gave satisfactory control in only 1 of 3 tests. Bayer 29493 gave 100% control as a 0.25% spray but did not give satisfactory control at concentrations of 0.1 and 0.05%. A series of tests was conducted to compare the effectiveness of Famophos, Bayer 37342, Bayer 29493 and Ruelene at different dosages on a "per animal" basis rather than on body weight estimates. In these tests minimum dosages of Famophos at 10 grams per head, Bayer 37342 at 5 grams per head, Bayer 29439 at 2 grams per head and Ruelene at 2 grams per head provided 98-100% control of grubs in cattle of mixed sizes and ages. These results indicate that dosage applied on a per-animal basis is as satisfactory as dosages applied on body weight estimates.

- 8. Horse Flies and Deer Flies. In Mississippi tests were conducted to determine the effectiveness of synergized pyrethrum dusts containing 0.1 and 0.067% pyrethrins. Both formulations gave excellent protection from horse flies the day of treatment and fair protection for several days. Mist spray applications of 2 ounces of activated pyrethrum containing 0.62% pyrethrins were highly repellent to horse flies for 6 hours. Lower concentrations were proportionally less effective. Similar applications of 5% ENT-21195 provided complete protection for 6 hours and afforded 79% protection after 24 hours.
- 9. <u>Lice</u>. In field tests in Mississippi, eradication of cattle louse infestations was achieved with a single spraying of 0.5% General Chemical 4072 and 1% Sevin. Similar results against hog lice were obtained with sprays of 0.1 and 0.25% of GC 4072, 0.5 and 1.0% of Sevin, 0.25% of Ciodrin and 0.5% of Ciodrin, Dilan, and methoxychlor.
- 10. <u>Ticks</u>. Research on the control of ticks was confined to the Texas laboratory. Only 5 of 79 compounds screened for systemic effectiveness showed systemic action against lone star ticks engorging on treated guinea pigs. The effective materials, dosages (mg./kg.) and routes of administration were as follows: Monsanto CP-19203, 10 mg. 0; Hercules 7845 and Stauffer R6032, 50 mg. 0 and SC; Shell 8280, 50 mg. SC; and Shell 8530, 100 mg. 0.

In field tests, sprays of 0.5% toxaphene and 0.25% Stauffer 1504 gave excellent control of lone star ticks on cattle and were slightly better in both immediate and residual effectiveness than 0.025% diazinon, 0.05% carbophenothion, 0.1% Ciodrin and 0.25% Sevin and Dilan. In tests against the winter tick, complete control was obtained with sprays of 0.05% carbophenothion, 0.25% Bayer 37341, 0.1% Dipterex, 0.25% Dowco 175, and 0.3% Ciodrin. Diazinon at 0.05% gave excellent but incomplete control.

C. Insecticide Residue Determinations

1. Residue Studies. Studies were conducted in Texas on the absorption, distribution, storage and metabolic fate of insecticides in animals using chemical and radiometric methods of analysis. In Texas, steers were sprayed with 0.05 and 0.1% diazinon 1, 2, 6, and 10 times at weekly intervals and residue determinations made 6 days after the last spraying. One and two sprayings of 0.05% diazinon produced residues of only 0.05-0.09 p.p.m. in the fat. Six and 10 sprayings produced residues of 0.20-0.35 and 0.17-0.23, respectively. However, one day after the 10th spraying residue levels ranged from 0.53 to 0.85 p.p.m. Animals sprayed with 0.1% diazinon showed residues ranging from 0.47 to 0.83 p.p.m. There was very little difference in the residue levels created by 1, 2, 6, and 10 sprayings.

A Hereford calf was sprayed with C^{14} -labelled Stauffer R-1504 at a rate equivalent to 2 quarts of 0.5% Stauffer R-1504. The calf was slaughtered 7 days after spraying and samples of tissues were taken for analysis. Analyses were made by a radiometric method and by the chemical sulfide method. Residues found by the sulfide method were 0.04 p.p.m. in the omental fat, 0.11 p.p.m. in the subcutaneous fat, and less than 0.02 p.p.m. (sensitivity of method = \pm 0.02 p.p.m.) in other tissues. The radiometric method (sensitivity of method \pm 0.001 p.p.m.) gave values of 0.030 p.p.m. in the omental fat, 0.41 p.p.m. in the subcutaneous fat, 0.021 p.p.m. in renal fat, and 0.001 to 0.004 p.p.m. in the other tissues.

Residue determinations were made from 3 calves slaughtered 7, 16, and 28 days after spraying with 0.25% General Chemical 4072. The amounts of General Chemical 4072 found in the various tissues ranged from 0.004 to 0.0085 p.p.m. 7 days after spraying, 0.004 to 0.006 p.p.m. 16 days after spraying, and in all cases were 0.005 p.p.m. 28 days after spraying. A still unidentified material appeared in the liver of the calf slaughtered 7 days after treatment, and also in other tissues of the calves slaughtered 16 days and 28 days after treatment. It is possible that this material is a metabolite of General Chemical 4072.

2. <u>Toxicity Studies</u>. Work was conducted in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other materials. A summary of the results are presented. Detailed results will be given under Unit 2, Animal Diseases and Parasites (ADP a7-12, ADP a7-18, ADP a7-19, ADP a7-20, and ADP a7-23).

A colorimetric analytical method for studying the toxicology and presence in animal tissues of 2,4-D was successfully developed by using Carbon-14 labeled 2,4-D which could be determined with radiation detection instruments at each step of the developing technique. The analytical method which resulted is capable of detecting 0.05 parts per million of 2,4-D in animal tissue samples weighing 25 grams.

The ordinary feeding of Vitamin A to cattle increased their susceptibility to poisoning by Co-ral, particularly when they were also given phenothiazine drenches for internal parasite control. When impurities appeared in Co-ral, as they did during the fall of 1962, the toxicity was even greater. However, contaminated Co-ral, such as was credited with causing losses of cattle exceeding \$750,000 in value, could not be shown to produce poisoning in Kerrville cattle unless Vitamin A and phenothiazine were also used. These initial studies need to be followed with others to determine whether the effect is limited to Co-ral or also follows the use of other insecticides.

More important than the increased incidence of poisoning when the Vitamin A and phenothiazine are present is the type of poisoning produced. Whereas Co-ral can poison and kill any animal, its activity normally is against an essential enzyme, cholinesterase. Poisoned animals that do not die rarely show any appreciable tissue changes. In the poisoning observed this year, there was a marked tissue change. One of the changes noted was a necrosis (death) of muscle fibers, particularly of the thigh muscles that compose the "round" meat cuts. In the living animal the necrosis is apparent as a lameness, in the carcass as an area of "white muscle" showing clearly against the normal red.

Four important enzyme systems were affected by the Vitamin A - phenothiazine - Co-ral combination. The significance of the effect is not yet clear.

Brahman cattle, and their crosses, were more susceptible to poisoning by Ciodrin and Compound 4072 than European breeds and their inter-crosses. The blood enzyme, cholinesterase, was more readily inhibited in Brahmans and their crosses than in European breeds. With Compound 4072, the only chemical given both by mouth and as a spray, the susceptibility was greater in Brahmans by both routes, indicating that the peculiarities of the Brahman skin were secondary to the species difference. The increased susceptibility was most marked to the compound Ciodrin.

In studies of the detoxication mechanisms in cattle and sheep, oximes, including 2-PAM chloride, DAM, and P₂S, were used to counteract poisoning by various organophosphorus compounds. The oximes are useful to cause a release of the enzyme (cholinesterase) inhibited by this group of pesticides. All three compounds were effective in mild or moderate poisoning. Their action was somewhat slow and atropine sulfate was still required in severe poisoning to gain time for the oximes to work.

Sodium selenite, sodium selenate, and d-alpha tocopheryl (Vitamin E) were effective in several instances of organophosphorus poisoning in our research. Studies are needed to explore the mechanisms by which these two substances exert their beneficial effects.

Studies of lindane sheep dips revealed that improper physical formulations were being employed, permitting the first sheep dipped in a fresh vat to

so deplete the fluid that the sheep dipped later were receiving extremely small concentrations in so far as control of parasites such as the scabies mites was concerned. The excessive amounts taken out by the first sheep dipped undoubtedly caused them to develop tissue residues far in excess of those normally to be expected.

Residues of 2,4-D in sheep fed the compound at a rate of 2 grams per head per day at Logan, Utah, were determined at Kerrville, Tex., using an analytical technique developed by the Kerrville staff. In sheep fed 30 daily doses, kidney, rumen, renal fat and body fat samples showed less apparent residue than did a control sheep. Muscle samples averaged less than 0.3 p.p.m. and liver samples less than 1.0 p.p.m. of 2,4-D.

In animals killed by the insect chemosterilants apholate, aphoxide, and methaphoxide, cytological changes were most prominent in the organs engaged in formation of the white cells of the blood. The changes indicated a severe decrease in ability of the parent tissue to supply the needs of the animals. In other studies, these materials were found to be highly cumulative in effect. Although some studies using low-level feeding are still current and the animals still alive, every dosage thus far tried has ultimately killed every sheep treated, in some cases after one year or more of exposure during which no observable illness occurred.

Thirty-two insecticides, most of them currently under test against live-stock insects, were studied during the year. These studies furnished toxicological guidelines for decisions for further development of the materials. Co-ral and arsenic did not show potentiation in cattle and calves when used on the same day or within one or two days of one another. The study was conducted to show the safety of using both compounds on cattle imported from Mexico. Sprays of toxaphene following Co-ral sprayings seemed to reactivate the Co-ral deposits, leading to mild Co-ral poisoning in cattle and calves so treated.

Three fungicides and thirteen herbicides were studied in cattle. Generally, except for the mercurial fungicides, massive dosages repeated on several days were required to produce poisoning. Four herbicides, simazine, atrazine, bandane and promazine afflicted the nervous system. With bandane, a yearling steer died of a cerebral and medullary hemorrhage after showing various degrees of paralysis and other neurological symptomatology.

D. Biological Control

1. <u>Mosquitoes</u>. At Fresno, Calif., in cooperation with the Bureau of Vector Control, California State Board of Health, studies were initiated on the biological control of mosquitoes. Several species of microsporidia of the genus <u>Thelohania</u> killed mosquito larvae. Studies are under way to determine the host-parasite relationship.

- 2. Stable Fly and House Fly. Spalangia muscidarum, a pupal parasite that attacks stable flies, house flies and other Diptera, was reared in sufficient numbers in the laboratory at Lincoln, Nebr., to permit the start of systematic releases on May 16. An area of 16 square miles containing 20 farms was selected as a release area and 5 farms at another location were used for control observations. The release rate was varied from farm to farm according to the extent of the potential fly breeding areas. The release of this parasite, plus the natural occurrence of several other parasites, failed to produce marked reductions in stable fly or house fly populations. The percentage parasitism of pupae was erratic but at times very high. In general, planted pupae were more heavily parasitized than naturally occurring pupae. The percentage parasitism ranged from 0 to 100, without any apparent correlation with either time of year or the number of parasites released. In the course of these studies it was determined that three parasites parasitize stable fly pupae only, four parasitize both stable fly and house fly pupae, and four parasitize house fly pupae only.
- 3. Face Fly. In Nebraska observations were made on the effect of dung beetles on face fly larval breeding in cattle droppings in pastures. The constant movement of the beetles through fresh droppings reduced larval populations by about 80%.
- E. Insect Sterility, Attractants and Other New Approaches to Control
- 1. Mosquitoes. In Oregon tepa and ENT-50450 were tested as sterilants against Culex tarsalis adults by spraying in wind tunnel tests and against larvae by exposure in water. Tepa sterilized males and females at a spray concentration of 6%; ENT-50450 sterilized only males at this concentration. Lower concentrations of either compound were not effective. Against larvae, both compounds were toxic at higher test concentrations and ineffective and partial toxicants at lower concentrations.

In Oregon studies have shown that grass infusion and log pond waters are attractive to ovipositing female Culex pipiens quinquefasciatus. Distilled water treated with methane or furfural was more attractive to ovipositing females than distilled water, but less attractive than log pond water.

2. Stable Fly. In Texas a small number of chemicals was evaluated by several methods as chemosterilants against the stable fly. Topical applications of 1.0 µg of ENT-26382 sterilized both sexes of flies. Similar applications of 4 µg of ENT-50569 sterilized males but did not completely sterilize the females. Three other materials—ENT-50042, 50396 and 50549—at 4 µg per fly, reduced oviposition and hatch but did not fully sterilize either sex.

In feeding tests, flies were completely sterilized when fed baits containing 0.2% aphoxide for 24 hours. Flies fed 0.1% aphoxide bait produced

normal numbers of eggs but only 2% hatched. Several materials were tested as residual treatments but none of them produced complete sterility.

Studies were conducted to determine the absorption, metabolism, and excretion of a P^{32} -labeled chemosterilant (MAPO) applied topically and in the diet of stable flies. When applied topically, the material was rapidly absorbed, with the females showing maximum absorption in 6 hours and the males in slightly longer time. The material was metabolized to the extent of about 60% in 24 hours. The main metabolic products were phosphoric acid and an unknown intermediate. Four other metabolites were isolated but not identified.

In the feeding tests MAPO was absorbed and distributed slower and in lesser amounts than in the topical tests. After 24 hours the degree of metabolism was about 50% higher than in the topical tests. In contrast to the topical tests, the main metabolite was an unknown intermediate which was about 4 times as abundant as phosphoric acid. Three of four other unknown intermediates occurred in greater amounts than phosphoric acid. Only about 8% of the applied dose of MAPO was excreted.

3. Face Fly. In Nebraska improved techniques were developed to screen materials as attractants for the face fly. Over 200 inorganic and organic materials were tested but none was as attractive as the liquid portion of fresh cattle manure.

A number of known chemosterilants was evaluated for effectiveness against the face fly. Flies consuming food containing 0.25% aphoxide for 24 hours were completely sterilized. Feeding for 3 days on a diet containing 0.0025% aphoxide prevented or greatly reduced oviposition and none of the eggs hatched. Males fed for 3 days on 0.005% aphoxide were incapable of fertilizing normal fertile females. Longevity of adults was not affected in these tests. ENT-26398, 50106 and 50107, at 1% in the diet produced complete sterility but were only partly effective at 0.25%.

4. Horn Fly. In Texas horn flies were sterilized by feeding overnight on a bait containing 0.05% tepa but a concentration of 0.01% was ineffective. Topical applications of 0.1 ug/fly fully sterilized both sexes of flies.

In Oregon adult horn flies were sterilized by exposing the pupae to 5000 r.

5. Screw-worm. In Texas where studies with the screw-worm were conducted, 57 of 350 compounds screened as candidate chemosterilants caused sterility when administered as topical treatments or fed to adults. Some of the compounds sterilized by both methods of administration. Secondary tests showed that some of these compounds sterilized one or both sexes completely, while others induced only partial sterilization or were ineffective. Tests with tretamine applied topically showed that adult flies could be sterilized when they were 1, 3, and 5 days old with equal facility. Males sterilized with thiotepa survived as well as untreated flies and competed

equally with untreated males in mating with females. However, the treated males were not as sexually competitive as untreated males. When a single dose of thiotepa or tretamine, adequate to sterilize either sex, was given in two half-doses 24 hours apart, survival of the flies was not improved and, further, a loss in sterilizing effectiveness occurred. Tretamine and thiotepa completely or partially sterilized screw-worms when puparia were immersed in solutions containing these compounds or were injected with them. With immersion, washing of puparia decreased the sterilizing effect indicating that adults obtained some or most of the sterilizing dose as they emerged from the puparium. Aerosol treatment of screw-worm adults with tretamine resulted in almost complete sterility.

Preliminary laboratory experiments indicated that ENT-50450 was as effective as gamma radiation in sterilizing screw-worm flies, and was superior in its lack of toxic side effects. Females mated to males sterilized with ENT-50450 continued to lay infertile eggs after the initial deposition of eggs indicating that sperm in spermathecae of females did not recover fertility. Males sterilized with ENT-50450 remained sterile throughout their lifetime.

Investigations with screw-worm flies and aziridinyl-type chemosterilants resulted in the conclusion that the primary influence of aziridinyl compounds on the ovaries of flies 0-4 hours old is the inhibition of oogensis, and of flies 1 day old, the induction of mutations. The effects of the aziridinyl compounds on the reproductive potential of female screw-worm flies were similar to those obtained with gamma radiation.

Studies were conducted to determine the absorption, metabolism and excretion of a P³²-labeled chemosterilant (MAPO) applied topically and in the diet of screw-worm flies. When applied topically the material was absorbed rather gradually. The absorbed material was metabolized to the extent of 42 and 58% in males and females, respectively, in 24 hours. The principal metabolic products were phosphoric acid and an unknown intermediate. Small amounts of five other intermediates were indicated but were not identified.

In the feeding tests, metepa was absorbed and distributed at about the same rates as in the topical tests. After 24 hours, the degree of metabolism in females was about the same as that in the topical test but, in the males, metabolism was 50% higher than in the topical tests. As in the feeding tests, the main metabolic products were phosphoric acid and an unknown intermediate. Five other intermediates were isolated but not identified. Approximately one-fifth of the applied dose of metepa was excreted.

Approximately 200 chemicals and other materials were screened as attractants for screw-worm flies. Of these, 10 were equal to or better than the standard liver bait and require further evaluation. Preliminary studies have indicated that mutant strains of screw-worm flies may respond

differently from normal strains to attractants since black-mutant flies were not attracted to the standard liver bait.

6. House Flies. Research on the development of sterilization for the control or eradication of house flies has been continued in Florida and Oregon. In Florida 886 new chemicals were screened for sterilant activity. Of these some produced toxic effects; however, 90 caused complete or partial sterility in the treated house flies. Seven compounds were highly effective in sterilizing both sexes of house flies, but two of these were also toxic at the dosages tested. Metepa and tepa as residual deposits on glass sterilized house flies at dosages from 25 to 250 mg. per sq. ft., but 5-fluorocrotic acid was ineffective as a residual deposit at similar dosages. Apholate in the adult food sterilized house flies regardless of their age and the flies did not regain fertility. Motile sperm were present in the testes of chemosterilized males and transferred during copulation to the spermathecae of females throughout the life span of the male.

Tests were initiated in Florida to determine whether a dosage of chemosterilant too low to prevent hatching or adult emergence might by the accumulation of small genetic injuries eventually reduce or eliminate reproduction. With apholate, one colony showed reduction of oviposition in the 4th, 5th, 6th, and 7th generations and no individuals reached the pupal stage in the 7th generation. With metepa, the 5th through 9th generation of another colony showed reduced oviposition and the 10th generation failed to oviposit.

Preliminary tests with apholate, tepa, and metepa, on house flies indicated that the probit of the percent sterility with house flies can be related to the log of the concentration.

Metepa and apholate shortened the life span of adult house flies considerably, but 90% or more of the male population survived the first 10 days, or that period of time in which mating activity is the greatest. Survival during the first 10 days was essentially the same in treated and non-treated house flies. Dipping house fly pupae in solutions of tepa, apholate, or metepa was toxic to most individuals, but a high degree of sterility occurred in individuals surviving the treatment.

Effective formulations of both dry baits and paint-on liquid baits have been developed.

A method has been devised for studying chromosomes of house flies. Using this technique, the effect of chemosterilants on spermatogensis and oogenesis will be made.

Studies conducted in Oregon with an olfactometer and with simulated treated fly models (pseudo flies) demonstrated the presence in female house flies of a volatile chemical or chemicals which can influence the

behavior of male flies. The behavior modification elicited was in the nature of attraction to a source of the pheromone, or an excitation of mating behavior patterns. The material which is benzene soluble and relatively stable was shown to be sex related, and appeared to be specific to the house fly since extracts of neither the face fly or stable fly affected the behavior of male house flies.

In Oregon tests were conducted to determine sterilizing effects of tepa and metepa on the little house fly (Fannia canicularis). All flies feeding on bait containing 0.1% tepa for 4 days and 0.5% tepa for 2 days succumbed in 3 and 5 days, respectively, and none oviposited before death. Flies feeding on 0.05% tepa for 3 days survived normally but did not oviposit. Metepa at 0.5% was highly toxic to flies. Flies feeding on baits containing 0.05 and 0.1% metepa survived and laid a few eggs but none hatched. Longevity of flies exposed for 2 hours on 100 mg. per sq. ft. residues of tepa was greatly reduced and all flies so treated were fully sterilized. Flies exposed on residues of 1 and 10 mg./sq. ft. laid a few eggs but none hatched. These tests indicate that the little house fly is much more easily sterilized than the house fly.

Over 50 inorganic and organic materials were tested as attractants for the little house fly. None showed significant attractancy.

In cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions, studies were continued in new facilities at Beltsville, Md., to develop physical and mechanical methods of controlling house flies and other flies affecting cattle. Colonies of flies were established for conducting laboratory and outdoor cage studies. Studies to date have been concerned primarily with the attractiveness of various flies to different kinds and intensities of light. Black light ultraviolet radiation was attractive to both house flies and face flies during twilight periods. The use of fluorescent panels behind light sources increased their attractiveness.

- 7. Cattle Grubs. In Texas studies were made to determine the radiation dosage required to sterilize cattle grubs (Hypoderma lineatum). Complete sterilization was obtained by exposing pupae to 5000 r. A dosage of 2500 r sterilized the females but not the males.
- 8. <u>Ticks</u>. Extensive tests were conducted in Texas to determine the effects of different levels of radiation on different stages of the lone star tick. One series of tests with nymphs which had been engorged 2 weeks were exposed to different levels of radiation and the adults subsequently placed on hosts. Adults from nymphs exposed to 500 r or 1000 r engorged but produced no eggs. At a dose of 2500 r adults engorged normally and treated females mated with treated males did not oviposit. One of 8 treated females mated with normal males oviposited and some of the eggs hatched. When females and males treated with 5000 r were confined on hosts

neither sex engorged. Similar results were obtained with untreated males and treated females, but when treated males and untreated females were used, 2 of 3 females engorged and oviposited. One of the two egg masses obtained was not viable and the other showed only a partial hatch.

Exposure of newly emerged adult ticks to 1000 r and 2500 r did not affect engorgement and complete sterility was indicated in crosses of treated males and females and in crosses of untreated males and treated females. Untreated females mated with treated males produced eggs but none hatched. One of the ticks exposed to 5000 r engorged but it did not oviposit. No ticks engorged after treatment with dosages of 7500 r or 10,000 r.

Some of adult ticks dipped in concentrations of 0.25 and 0.5% of apholate, tepa, metepa, or tretamine were killed but those that survived engorged and laid viable eggs. Dipping in 1% concentrations of these materials also failed to produce complete sterility but the maximum viability of eggs from females treated with apholate was only 0.25%.

F. Evaluation of Equipment for Insect Detection and Control

1. In Texas, in cooperation with the Agricultural Engineering Research Division, studies were undertaken to develop control mechanisms for automatic spraying devices that would be stable and dependable and which would not excite cattle. Step-on electric switches (switch-mats) installed on the ground underneath the spray nozzles were superior to the usual electric eye switches. The step-on switches provided quick and reliable operation of sprayers and required only limited maintenance. Cattle were not aware of the imperceptible movement when they stepped on the mat and activated the sprayer. Most of the commercially-available automatic sprayers have mechanical defects and several adaptations were tried as improvements. One adaptation of the ring-type fitted with 7 nozzles proved superior to the commercial types.

At Beltsville, Md., in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions, work was initiated to develop test equipment and techniques of operation to evaluate the attractiveness of visible and ultraviolet radiation to house flies or other flies affecting cattle. This research has not yet reached a point of profitable summation.

G. Insect Vectors of Diseases

1. Anaplasmosis. Studies were continued in Texas, Mississippi, and Oregon to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. These studies were conducted in cooperation with the Animal Disease and Parasite Research Division and veterinarians of the various State experiment stations. In Texas, monthly surveys were continued to determine the abundance and identity of external parasites on infected (anaplasmosis) and clean herds

of cattle. Lone star ticks were abundant on cattle during May, June, and July, 1962, but declined rapidly in August and partially disappeared by September. During this same time, ear tick populations were high except for August. Horn flies became numerous early in May but periodical spraying of cattle minimized populations the remainder of the season. The winter tick and black-legged ticks appeared in November. The winter tick was abundant by December, but black-legged tick populations remained low. Moderate populations of the ear tick were present from October through December. Small numbers of lone star and black-legged ticks and large numbers of ear and winter ticks were present on cattle in January, 1963. In February and March, 1963, populations of the lone star tick increased, while the winter and black-legged ticks virtually disappeared. Ear tick populations remained high.

Prophylactic treatment with aureomycin minimized transmission of anaplasmosis and prevented acute cases. All of the 10 animals in the control herd contracted the disease and 3 of them had acute cases. At the low level (2 mg.) the maximum number of negative animals occurred after 3 months of treatment, whereas at the high level the maximum number occurred 3 months after treatment was terminated. In time, most of the negatives reverted to positives. The period of greatest transmission of the disease coincided with that in which the horse flies, Tabanus lineola and T. vittiger schwardti, and the mosquito, Psorophora confinnis, were most abundant.

In Maryland, hereditary transmission experiments and histopathological studies with the vectors of anaplasmosis of cattle were continued in cooperation with the Animal Disease and Parasite Research Division. Two series of transmission trials with the vector, Dermacentor andersoni, were conducted. In one, the F1 larval and adult progeny of female ticks fed on an animal in the acute stages of anaplasmosis failed to transmit anaplasmosis to test calves. In tests with larval progeny, challenge of the animals with the disease proved the animals to be susceptible to the disease. Proof of the susceptibility of animals used with adult progeny has not been completed. In another series of trials, unmated and mated males fed on an animal with acute anaplasmosis were studied for survival under hibernating conditions (4-5°C. and 30-50% relative humidity with a photoperiod of 7 hours) and their ability to transmit anaplasmosis. Mated males survived only about 4 months in the hibernating environment and transmission trials could not be run. Eleven of 49 unmated males survived the 8-month-hibernating period, and 6 of these survived for transmission trials 3 months after the hibernating period. None of the survivors transmitted anaplasmosis to test cattle; however, not all of the animals have been proved susceptible by challenge at this time. In similar studies previously reported, transmission by infected males was demonstrated.

In Maryland, histopathological studies on Anaplasma-infected and non-infected Dermacentor andersoni were continued. Using immunofluorescence methods, anaplasmata were found in gut and excreta smear preparations from

ticks fed on infected animals. Brightfield and electron microscopic examinations revealed anaplasmata in excreta. Electron microscopic studies showed structures believed to be A. marginale in undigested erythrocytes. However, anaplasmata were not found in ultrathin section of the salivary glands and reproductive organs.

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EQUIPMENT AND BUILDINGS USED IN PRODUCING BEEF CATTLE Agricultural Engineering Research Division, ARS

Problem. The American farmer has about \$14 billion invested in service buildings and related structural equipment, over half of it for livestock facilities. Maintenance and new construction amount to another \$1.2 billion annually, again mostly for livestock facilities.

Economic conditions are forcing changes in the pattern of livestock production. Producers are trending toward fewer, larger and more specialized enterprises and toward "confinement" types of facilities in their effort to reduce production costs and improve product quality. These trends are demanding more basic knowledge on the effects of environment on the health, growth, production and fertility of livestock; on structures and related equipment for maintaining optimum environments; and on methods, structures and equipment for more efficient handling and feeding. The continuing threat of nuclear warfare demands consideration of types of buildings that could provide protection from fallout for livestock and their feeds, and provide facilities for operation during periods of emergency.

Much more needs to be learned in the laboratory on the relationships between livestock environment and disease transmission, feed conversion rates, and growth and production in order to determine optimum environments. Structures and equipment for economically providing these optimum environments under practical conditions need to be developed and field tested. Closely associated with the environment are flies and other insects, as well as parasites and diseases, that sap the vitality of animals and reduce their productivity. Pesticide residues in animal products are causing much concern. Information is needed on means for keeping these residues from adversely affecting the animals or their products.

Labor also is an important element in overall production costs, and if only family labor is available, the labor requirement limits the size of enterprise. How to adapt existing buildings and other facilities for more efficient production, as herds and flocks are increased in size, or as farms are consolidated, is a major problem area. Cost of replacement or major improvement of existing buildings that are not suited to modern production methods are serious obstacles. Principles, examples and techniques for planning more efficient operations are needed both by farmers doing their own engineering and by those on whom farmers depend for advice.

Today's technology in farming requires accurate instruments for measuring or monitoring processes such as grain and forage drying and plant and animal environment. Some problems require completely new kinds of instruments. Studies are necessary to determine the accuracy and practicability of instruments for many kinds of agricultural measurements.

USDA PROCERAM

Research pertaining to livestock engineering is a continuing program involving engineers and architects conducting basic laboratory investigations, application of laboratory results to a production basis, and development of typical plans for livestock structures. The work is in cooperation with the AH, ADP, and ENT Divisions of ARS, USDA, and State Agricultural Experiment Stations. Plan development work is cooperative with all the State Agricultural Experiment Stations and Extension Services.

Beef cattle structures and equipment research for hot, dry climates is conducted in cooperation with the California Agricultural Experiment Station at the Imperial Valley Field Station, El Centro. Related studies for a warm humid climate are in cooperation with the Missouri Agricultural Experiment Station at Columbia and with AH, ARS. Typical plans for beef structures are developed at Beltsville.

Shades for sheltering livestock are being studied at Tifton, Georgia, in cooperation with the Georgia Station.

Reduction of pesticide residues in animal products, with beef cattle receiving major attention, is studied at Kerrville, Texas, in cooperation with ENT and ADP, ARS, and the Texas Agricultural Experiment Station.

Federal research in the area of livestock engineering totals 9.1 professional man-years. Of this number 2.2 is devoted to dairy; 0.3 to beef; 1.3 to swine; 3.2 to poultry; 0.1 to shades and shelters; 0.2 to sky radiosity studies; 1.0 to reducing pesticide residues in animal products; and 0.8 to program leadership.

Equipment and control for automatic feeding of livestock and poultry is under development in Washington and Illinois State Experiment Stations. Work on performance characteristics of upright-silo unloaders is in cooperation with the Minnesota State Experiment Station. Federal effort devoted to research on electric equipment for farm labor reduction amounts to 5.0 professional man-years, of which 2.5 are for livestock and poultry.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Beef Cattle Engineering

1. Hot, arid climate. At El Centro, in the Imperial Valley of California, in cooperation with the California Station, four pens of beef cattle were used to compare the effects of a cooled shade, sprays, and spot refrigerated coolers, with a typical shade. The cooled shade was constructed under one of the regular shades. The sub-roof was made of excelsior pads supported

on chicken wire. Spray nozzles were placed above this and their flow was regulated by a time clock and solenoid values. A plenum chamber was made by enclosing the sides with plastic film. Two large, slow-speed fans forced air into this chamber, past the sprays and through the excelsior onto the cattle. In another pen, two 1-hp commercial air coolers were mounted so that the air stream from one, about 500 cfm, blew across the backs of the animals; the stream from the other cooler blew down onto the animals. Two other pens had two sprinklers in each. Water flow was timed so as to minimize muddy conditions under the shades. Hereford yearling heifers were used in a feeding trial from July 18 to October 31, 1962. The weight gain and feed conversion are shown below:

	Controls (shade only)	Water Sprays	Evaporatively Cooled Shade	Refrigeration Units
Number of animals	6	6	5	5
Initial wt. lb. Daily gain, lb. Gain/100 lb. feed	60.4 2.10 11.4	60.7 2.04 11.5	81.0 2.03 10.9	85.1 2.40 12.2

There were no particular benefits from any treatments other than the refrigeration units. Observation indicated the animals preferred to use the unit discharging downward. Feed consumption and daily gains were increased over control animals although differences in energy gain or corrected carcass weight were not significant.

- 2. <u>Hot, humid climate</u>. At Columbia, Mo., in cooperation with the Missouri Station, fifty-four grade Hereford heifers were randomly placed in four pens with the following treatments:
 - (1) Control inside and outside pens constructed as other three except radiation panels were not cooled.
 - (2) Condensate drip from cooled panels channelled to outside drain.
 - (3) Condensate drip allowed to fall into pen.
 - (4) Same as (3) but animals allowed free access to outside pens.

Average daily gains for the ll-week period were: (1) 1.28 lb.; (2) 1.42 lb.; (3) 1.39 lb.; and (4) 1.37 lb. Providing radiation cooling for growing beef calves did not markedly improve gains although a trend favorable to radiation cooling was apparent. However, the animals were small and were on growth rations. Future tests would be more meaningful with fattening animals.

3. Plan development. At Beltsville, Md., a plan was developed for a combination hay storage and feeding shed suitable for dairy and beef cattle use throughout the South (and to some extent in other regions). A plan was also developed for a cattle feeding shelter suitable for the South and West and having covered feed bunks with poles in the center so that an auger can work between the poles or a self-unloading wagon can empty directly into the bunk. These plans were developed for the Cooperative Farm Building Plan Exchange.

4. Livestock Shades and Shelters

At Tifton, Ga., in cooperation with the Georgia Station, a study was continued in an effort to define the best height for cattle shades in a hot, humid climate. Observations in 1960 indicated an advantage, in terms of lower black globe temperatures at animal level, for a 6-ft. shade compared to others 9 and 12 ft. high. During a limited series of tests the past year, no differences were noted. This test will be repeated with the inclusion of directional radiometer measurements.

5. Reducing Pesticide Residues in Animal Products

Field tests of experimental automatic walk-through sprayers for controlling flies on cattle, conducted at Kerrville, Texas, in cooperation with the Entomology Research Division, indicated that they controlled horn flies on cattle as well as did power sprayers used in pens, and used 37% less insecticide. The insecticide used was one of the two that can be sprayed directly on lactating cattle without leaving residues in the milk. The sprayers appeared to be suitable for herds that must come to specific watering spots in their daily routine or that must be milked (in the case of dairy cattle). Operation of the sprayers during the field tests indicated that reliability without supervision and speed of operation are factors which must be optimized for the most efficient use.

In developing and evaluating equipment and procedures for reducing chemical hazards associated with the control of livestock insects a measure of the amount and location of the spray applied to each animal is very important. At Kerrville, Texas, methods of quickly and accurately measuring the spray retention have been evaluated and a number of them discarded. The use of a fluorescent dye tracing technique is presently being studied as a means of measuring both distribution and retention. Preliminary trials indicate that only 38% of a one-gallon spray of clear water will remain on a mature Hereford cow when the application is made with a power spray.

6. Physical Methods of Fly Control

Investigations of physical methods for controlling flies in and around dairy barns are being conducted at Beltsville, Maryland, in cooperation with the Animal Husbandry and Entomology Research Divisions, ARS. Construction of a new laboratory building for this research was completed in November 1962 at Beltsville, Maryland. Preliminary studies conducted in temporary facilities at Orlando, Florida, and at Beltsville indicated that blacklight ultraviolet radiation is attractive to both house flies and face flies during twilight periods. Use of fluorescent panels behind the light sources appeared to increase attractiveness.

A successful colony of face flies has been established. Studies are now being conducted to determine the effect of colony illumination levels on face fly egg production, period for development, adult longevity, and adult behavioral responses. Observations were made on the behavior of face flies in the field during twilight to determine characteristics which might be useful in applying controls. Face flies were marked, released, observed, and some relocated after sunset. Test equipment and techniques are being developed to evaluate the attractiveness of visible and ultraviolet radiation to face flies, house flies, and stable flies.

B. Cattle Feeding Equipment

1. Beef cattle equipment. In Illinois, work, cooperative with the University of Illinois Agricultural Engineering Department, has progressed on an automatic cattle feeding system. The current-sensitive silo unloader control has been proved to be sufficiently accurate for normal cattle feeding operations. A cable supported top-unloading silo unloader equipped with two motors, one to power the gathering auger and one to power the blowerthrower, will deliver grass or corn silage at a constant rate within plus or minus 5 percent of the set amount in pounds per minute. This controller is equally adaptable to single motor unloaders for the purpose of keeping the unloader motor properly loaded. An electric controls manufacturer is developing a control system for silo unloaders using the current sensing method. Also in Illinois a variable speed auger is being used to meter high moisture shelled corn. The variable speed auger removes shelled corn in direct proportion to the auger speed. The final metering rate in pounds per minute is affected by the percentage of fines in the high moisture shelled corn because a high percentage of fines impedes the flow of corn to the metering auger. Power consumption by the meter auger varies widely depending upon the amount of fine material in the shelled corn. A tapered inlet screw 31-inches long was used to load the 5 1/2-inch discharge and metering auger. The auger was kept 60 percent full with this arrangement. At 10 to 100 revolutions per minute the 5 1/2-inch auger discharged an average of 1.31 pounds of high moisture shelled corn per revolution. An average input of .0086 hp. per revolution per min. was required to turn the auger. This was subject to considerable variation. Automatic operation of a sweep auger in the high moisture shelled corn is essential for reliable operation of the metering and discharge auger.

2. Sile Unloaders. In Minnesota the performance of electric motors for \$110 unloaders is being determined in cooperation with the University of Minnesota Agricultural Engineering Department. Two makes of standard late model unloaders were installed in two concrete stave silos filled with wet corn silage. The moisture contents of the silages ran 81 percent and 83 percent on a wet basis. The silages on top of the silos were slightly dried out and the unloaders were able to unload the material. At a depth of five feet from the top, both units ceased to deliver any silage. Power demands by this time had doubled. The speeds of the blowers in both units were reduced by 30 percent and unloading again was possible. The speed reduction and a slight blower housing modification by the manufacturer made possible complete unloading of one silo with one make. In the other unit impeller blades of a different design were installed in addition to the speed reduction to enable complete unloading of the silo.

Performance tests of specially designed capacitor motors would indicate ability to operate unloaders if adequate voltages are maintained. One of the repulsion induction motors now in common use did not meet the nameplate ratings for duty cycle. Use of a silo unloader to feed an Experiment Station beef breeding herd reduced labor costs by 50 percent when the silage was frozen and by 70 percent when it was not frozen.

In Washington an automatic horizontal or trench silo unloader is being developed in cooperation with the Washington State University Agricultural Engineering Department. The cutter unit developed in 1962 was tested with two 32-inch long, 16-inch diameter augers. These larger augers were more effective than the previous 12-inch diameter augers, removing more than 600 lb./min. of peavine silage. With an imput of 2 horsepower the cutter will deliver more than 400 pounds of peavine silage per minute. The major work now in progress is the incorporation of the cutter with suitable conveyors to remove the silage from the silo. This has resulted in the development of an experimental unloader. The present unloader design requires 7 3/4 horsepower. When operational difficulties have been overcome, automatic controls will be added.

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II. NUTRITION, CONSUMER AND INDUSTRIAL USE RESEARCH

NUTRITION AND CONSUMER USE RESEARCH

Consumer and Food Economics Research Division, ARS
Human Nutrition Research Division, ARS

Problem. The assortment and characteristics of foods available to consumers are constantly changing with the adoption of new production, processing, and marketing practices. Constantly changing also, as nutrition science advances, is our understanding of the nutritional needs of man and the manner in which these needs can best be met by food. To help carry out the Department's responsibility to advise on the quantity and variety of foods that will assure maximum benefit and satisfaction to consumers, continuous research is essential on the nutritional requirements of persons of all age groups, and on the nutrient and other inherent values of foods and how to conserve or enhance these values in household preparation and processing. Periodic examinations of the kinds and amounts of foods consumed by different population groups and individuals also are essential for evaluation of the nutritional adequacy of diets and to give the guidance needed for effective nutrition education. Such information provides assistance needed in market analyses for different commodities and in the development and evaluation of agricultural policies relating to food production, distribution, and use.

USDA PROGRAM

The Department has a continuing program of research concerned with (1) nutritive and other consumer values of raw and processed foods as measured by chemical or physical means and by biologic response; (2) effects of household practices upon the nutritive values and inherent qualities of foods, and the development of principles and improved procedures for household food preparation, care and preservation; (3) surveys of kinds, amounts, and costs of foods consumed by different population groups and the nutritional appraisal of diets and food supplies; and (4) development of guidance materials for nutrition programs.

The research is carried out by two divisions of the Agricultural Research Service--the Human Nutrition and the Consumer and Food Economics Research Divisions. Most of the work is done in Hyattsville, Maryland, and at Beltsville, Maryland; some is done under cooperative or contract arrangements with State Experiment Stations, universities, medical schools, and industry. The total Federal scientific effort devoted to research in these areas totals 66.3 man-years. It is estimated that approximately 7.7 man-years is concerned with studies related to beef and beef products.

Human metabolic studies and the related exploratory and confirmatory studies with experimental animals and microorganisms concerned with defining human requirements for nutrients and foods are not reported on a commodity basis, though some of the work is applicable to this report. This basic nutrition

research represents a total Federal effort of 23.4 professional man-years and is described in detail in the report of the Human Nutrition Research Division. Certain aspects of this research related to lipids are considered briefly in this report.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Nutrient Values of Beef

1. Tables of food composition. The 1963 revision of Agricultural Handbook No. 8, "Composition of Foods...Raw, Processed, Prepared" was completed and carried through to the galley proof phase. Data are included for about 175 beef items and beef products. For beef carcass, the composition of six market grades are shown. Retail cuts, trimmed to the retail basis, are reported for the two most important grades, choice and good, and for each grade as raw and as cooked meat. Data are given for separable lean and separable fat tissue so that these can be combined in any desired proportions for individual needs. This provides for variations from the average in retail trimming, and additional trimming in the home. Veal is classified by fatness rather than grade, with over 30 items included.

Data in the popular publication, "Nutritive Value of Foods," Home and Garden Bulletin No. 72, have been revised to agree on a weight basis with nutritive values in Handbook No. 8. The revised edition will provide nutritive values of household measures of 512 commonly used foods. Another popular publication, "Conserving Nutritive Value of Foods," Home and Garden Bulletin No. 90, is in press.

2. Fatty acids. The fatty acid composition of selected food fats has been determined using gas-liquid chromatography for separation of fatty acid esters. A manuscript was prepared presenting procedures of extraction resulting in minimal alteration in fatty acid composition.

Linoleic acid comprised 7.5 percent and linolenic acid 0.5 percent of the total fatty acids in mixed animal and vegetable shortening currently available in retail markets, and 6.8 and 1.0 percent respectively in a mixed pork and beef shortening. Research has been initiated to determine the fatty acid content and composition of raw and cooked ground beef and cooking drippings.

- 3. Proteins and amino acids. A manuscript was published describing a method for assay of alanine using <u>Leuconostoc citrovorum</u> 8081 and reporting results of analysis of 48 proteins and foods, including hamburger which contained 0.351 grams of alanine per gram of nitrogen.
- 4. Proximate composition of main dish meals. The proximate composition of 38 main dish foods in several market forms—home-prepared, packaged combination, canned, frozen and chilled—has been determined. These studies were carried out cooperatively with the Fish and Wildlife Service, U.S. Department

of the Interior. Plain meats, frozen dinners, sandwiches, pies, fried breaded foods and combination dishes were included in this study. Caloric values calculated for sandwiches, pies, and fried breaded foods were high. Combination dishes such as chow mein without noodles were low. For most foods, caloric values tended to follow fat content. Protein content ranged from 5 percent or less in spaghetti and commercial chow meins to more than 15 percent in most plain meats and fried breaded foods. Canned products differed more from their home-prepared counterparts than did frozen or chilled commercial forms. Commercially prepared foods generally had lower fat, protein, and caloric values, and higher ash and carbohydrate contents than did corresponding home-prepared foods. Composition values for most forms of foods were remarkably consistent among the lots analyzed. The results have been summarized and a technical bulletin prepared for publication.

5. Biological values. Lipid biosynthesis is being studied as a possible criterion for assessing the nutritional value of foods. Rats developed acute deficiency symptoms when fed a cholesterol-free diet plus an inhibitor of cholesterol biosynthesis. The content of total sterols in their carcasses and tissues was about the same as for control animals fed a cholesterol-free diet. Analysis of the major sterols by Entomology Research Division showed 75 percent of the total sterols to be desmosterol and less than 20 percent to be cholesterol in the carcasses of the inhibitor-fed rats; in the carcasses of control rats 95 percent of the total sterols was found to be cholesterol. A manuscript presenting these findings has been accepted for publication. In other phases of this research, lipid biosynthesis in relation to age and diet is being investigated.

B. Properties Related to Quality and Consumer Use of Beef

- 1. Shortening properties of fats. Investigation of the shortening properties of five kinds of fat--corn oil margarine, hydrogenated vegetable fat, hydrogenated vegetable and animal fat, regular margarine, and butter at different levels of added fat, liquid, and sugar in white cakes is in progress. Sensory, physical, and chemical measurements are being used to determine the influence of the proportion and kind of fat on the quality of the baked product. A report on levels of fats and oils in pastry and biscuits was accepted for publication in Cereal Chemistry.
- 2. Quality of cooked beef. Research on the cooking quality of beef differing in amounts of fatness and connective tissue is in progress. Laboratory work has been completed on steaks from the 9-10-11-rib cut and the eye of round from 20 beef carcasses. Data have been collected on the quality characteristics of raw steaks and on cooked steaks after broiling to internal temperatures of 140°, 160°, or 180° F. Correlations of palatability characteristics with physical and chemical determinations of fat, moisture content, and color will be investigated and related to observable characteristics. Results will be used to guide recommendations for improved methods of cooking.

3. Quality of freeze-dried meat products. Freeze-dried meat products were evaluated for general acceptability, appearance, flavor, juiciness, texture or consistency, and tenderness in cooperation with the Marketing Economics Division, Economic Research Service. The object was to provide information on which to base estimates of consumer acceptability of freeze-dried products in retail, institutional, and manufacturing markets, and suitability of these foods for specialized uses such as emergency rations, catering, and armed services use.

Food products included in a comparative study of freeze-dried foods and other processed forms of the same food were sliced beef and gravy, vegetable-beef stew, beef noodle soup, chili with beans, swiss steak dinner, diced beef, hamburgers, and beef steak. Either frozen or canned processed products were selected for comparison with the freeze-dried foods whenever possible. Fresh products were selected only if a processed one was not available on the local retail market.

All of the freeze-dried foods were considered acceptable in quality. The general acceptability of freeze-dried beef noodle soup was significantly higher than that for canned soup; the quality characteristics of flavor, texture, and appearance were not significantly different. Quality characteristics of freeze-dried diced beef, beef steaks, and sliced beef and gravy usually were rated lower than for the canned or frozen counterpart. The freeze-dried products given low scores were weak or lacking in flavor or off-flavored, fibrous, stringy, powdery, mealy or woody in texture, and tough and chewy in tenderness.

No significant differences were noted between freeze-dried and frozen hamburgers. The general acceptability of freeze-dried combination dishes was fair to good for vegetable beef stew and chili with beans, and poor to fair for swiss steak and peas.

Results of these studies are published in a marketing research report.

4. Fresh, frozen, canned meat products. Preparation time, serving yield, food components, and quality characteristics were determined for home prepared and purchased chilled, frozen, and canned meat products. Results have been summarized for publication as a Department bulletin along with similar data on poultry, cheese, and fish items. The lean meat content in frozen dinners or main courses ranged from 31 to 44 percent of the weight of the ready-to-eat food. The content of lean meat in frozen beef pie was 16 percent and in canned beef stew, 15 percent. The lean meat content in four brands of frozen beef pie varied from 15 to 20 percent of the weight of the ready-to-eat pie.

C. Nutrient Functions

Lipids. A better understanding of specific relations between diet, health and longevity has resulted from long-term investigations with

laboratory animals fed 29 different experimental diets including one that contained 25 percent beef and beef fat. Both excessive food intake and relationship or balance of nutrients in the diet are implicated in the adverse effects that occurred throughout the lifespan of laboratory animals. The studies indicate that genetic strain affects the response to the different diets and thus emphasize the importance of recognizing inherited characteristics in evaluating response to diets. Survival varied even with diets of similar fat and protein content. Differences in serum cholesterol levels of animals showed no relationship to kind or level of fat nor to level of dietary cholesterol.

D. Food Consumption and Diet Appraisal

1. Food consumption and dietary levels. A report of the findings of the food consumption survey of beneficiaries of Old Age and Survivors Insurance made in Rochester, New York in the spring of 1957 has been completed. The survey included 283 1- or 2-person households. During the survey week, food brought into the kitchens of these households averaged about the following amounts per person: 4 quarts of whole milk or its equivalent in milk products; 4 pounds of meat, poultry, fish; 1/2 dozen eggs; 10 pounds of vegetables and fruits; 2 pounds of grain products (in terms of flour); 1 pound of sugars and sweets; and 3/4 pound of fats and oils. The total money value of all food per person was \$8.12. Nutrients from this food more than met the National Research Council's recommended allowance for the average person. However, less than half (44 percent) of the households had diets which met in full the recommended amounts for all nine nutrients (good diets). Nearly three-fourths of the households had diets that met two-thirds of the recommendations for all nutrients (good and fair diets). The nutrients which fell below the recommended allowances most often were thiamine and calcium.

The series of food surveys conducted in low-income areas to aid in the study of the effects of food distribution programs on diets of families has been extended to include a survey carried out in Choctaw County, Oklahoma and in Pensacola, Florida. These were conducted cooperatively with the Marketing Economics Division, Economic Research Service as were similar surveys reported previously.

A food consumption survey was carried out in the District of Columbia that will provide information on the diets of households and of individuals. The study was undertaken primarily as a pilot survey in developing procedures for the next Nationwide survey proposed in the Department's long-range program.

The nutrient content of the per capita food supply is calculated and published each year, using data on estimated quantities of foods consumed (retail-weight basis) as developed by the Economic Research Service. This series, with estimates extending back to 1909, is the only source of data on year-to-year changes in the nutrient content of the U.S. per capita food consumption.

2. Food management practices. The results from three small studies based on records kept by the homemaker on the kind, amount, and nutritive value of foods used and discarded in households have been prepared as a journal article. In terms of total calories available for consumption, discarded edible food averaged 7 percent in St. Paul, Minnesota; 8 percent in DeKalb County, Missouri; and 10 percent in Los Angeles, California. A study using "recall questions," instead of records with a random sample of 300 households in Minneapolis-St. Paul in the winter of 1960 is currently being processed.

A report on household practices in handling and storing commercially frozen foods, based on surveys in two cities has been published. Survey findings indicate that household practices alone would not cause serious quality deterioration of frozen foods.

A new study has been initiated (under contract) of the management practices of urban and farm home freezer owners in Fort Wayne, Indiana and a nearby rural area. The survey is designed to obtain information on such actual management practices of home freezer owners as the kinds, amounts, sources, prices, and rate of turnover of foods frozen and stored in the home.

3. Development of food budgets and other basic data for food and nutrition programs. The ongoing program of interpretation and application of nutrition research findings to practical problems for use by nutritionists, teachers, health workers, and other leaders concerned with nutrition education or nutrition policies has involved the preparation or review of articles and publications, talks, television interviews, and participation in various conferences and committees.

With the publication of the report "Family Food Plans and Food Costs" the technical work on the development of the Department's current low-cost, moderate-cost and liberal food plans was completed. The continuing phases of the work on individual and household food budgets consists in the regular pricing of the food plans for publication in Family Economics Review, and in dissemination of information concerning them through such popular publications as "Family Food Budgeting for Good Meals and Good Nutrition," through filmstrips ("Food for the Young Couple"), and through correspondence, talks and committees (such as the Advisory Committee to the Bureau of Labor Statistics on their City Workers' Standard Budget).

Progress on the revision of Handbook No. 16, "Planning Food for Institutions" has focused primarily on the food purchasing guide section. Publications in preparation that are designed for the use of teachers, extension workers and other leaders are (1) a semi-popular publication on nutrition in the series Facts for Nutrition Programs; (2) a report on fat and related components in U. S. diets; and (3) a study of the relative economy of foods.

Nutrition Committee News, a bimonthly periodical prepared for members of State nutrition committees and other workers in nutrition education provides one channel for disseminating pertinent information and for reporting nutrition education activities. Examples of subjects of current interest covered during the report period are: "Nutrition Aspects of Selected Studies of Cardiovascular Diseases--Implications for Nutrition Education," "Planning Nutrition Programs for Elementary School Teachers," and "Food Guides--A Teaching Tool in Nutrition Education."

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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TII. MARKETING AND ECONOMIC RESEARCH

LIVESTOCK AND MEAT - MARKETING FACILITIES,
EQUIPMENT AND METHODS
Transportation and Facilities Research Division, AMS

Problem. Many of the livestock, meat, and wool marketing, slaughter, and warehouse facilities occupied today are obsolete and the work methods that can be used in such facilities are antiquated. As a consequence, labor costs are excessive and they are increasing. Many firms still are occupying facilities designed primarily for handling rail receipts and rail shipments even though the majority of these products today are moved by motor-This situation also adds to handling costs. Numerous firms are occupying "makeshift" facilities which were designed for other uses or for work methods and operations of a bygone era when labor costs were low. Changes in transportation systems, population growths and shifts, and advancements in technology also have brought about changes in the types of facilities needed - such as livestock auction markets, commercial feedlots, and hotel supply houses. Most private firms handling livestock, meat, and wool lack the technological and engineering skills necessary to plan and develop suitable facility layouts and designs and to select the types of equipment needed. Therefore, engineering and related research is needed to provide guidelines for industry to increase efficiency; including the designing of improved plant layouts, which will provide proper arrangement of work areas to minimize travel distances and excessive handling and the development of work methods that will permit use of mechanized and automated equipment rather than the relatively high-cost manual methods now used in many plants.

USDA PROGRAM

The Department has a continuing long-term marketing research program involving industrial engineers, agricultural economists, and meat scientists engaged in both basic and applied research to develop new and improved methods, equipment, processes, and facilities for livestock markets, meat packers and wholesalers, and wool warehousemen. Livestock market research is carried on at Washington, D. C. Part of the work in this area is being done either under contract or in cooperation with the Toledo Scale Corporation, Toledo, Ohio, and the Central Missouri Livestock Auction, Mexico, Mo. Work on the behavioral patterns of livestock is under a contract with the American Research and Mfg. Corp., Rockville, Md. The research on livestock slaughtering and on meat packing and wholesaling at Stillwater, Okla., is cooperative with the Oklahoma Agricultural Experiment Station. Wool warehouse research is carried on at Washington, D. C.

The Federal effort devoted to research in this area totals 6.3 professional man-years; 3.1 man-years (including 2.1 man-years of contract work) on live-stock marketing, 2.3 man-years on meat facilities, 0.2 man-year on wool warehouses, and 0.7 man-year on program leadership.

REPORT OF PROGRESS OF USDA AND COOPERATIVE PROGRAMS

A. Automation of Sales Operations on Livestock Markets

At the Central Missouri Livestock Auction Market, Mexico, Mo., the combination electronic load-cell and lever-system scale, and the scoreboard for flashing gross weight, average weight, and price to the audience continues to perform well. The manual-key input device for transmitting sales information from the auctioneer's box to the office and the computer for receiving sales information, making the necessary calculations, and preparing the seller's check have not proven satisfactory. The time required by the computing and recording systems tested results in a delay of the auction sale. To eliminate the delay, this equipment must perform its function in about 20 seconds per sale or transaction instead of the 35 seconds now required. At the end of the year equipment modifications were being made to speed up the cycle.

As previously reported, the electrically-operated pen gates; developed under a research contract with Milro Controls Company and installed on the Mexico, Mo., market; failed to function with the degree of satisfaction desired. Although the gates opened and closed at the proper speed and stopped when coming in contact with animals without injuring them, when once stopped by animals during the opening and closing cycle the gate could not again attain the desired speed for opening or closing promptly. A new contract was negotiated with the Toledo Scale Corporation during the year to determine the physical and economic feasibility of developing electrically-operated gates. The contractor's report is due on or before April 1, 1964.

B. Determining Behavioral Patterns of Livestock

A contract with the American Research and Manufacturing Corp., Rockville, Md., was negotiated for developing design criteria for an automatic driving device for livestock markets. This research will involve observations and experiments to establish the behavioral patterns of cattle, hogs, and sheep under environmental conditions existing on stockyards and auction markets when handled or driven both singly and in groups ranging up to 10 animals and when they are subjected to:

- 1. Light rays of different candlepower, intensity, and bands of the spectrum.
- 2. Sounds of different pitch and intensity.
- 3. Air blasts of different velocities and temperatures.
- 4. Electricity applied at different voltages and by various means.

A mechanical "sweep" or "driver," of alley or sales ring width, equipped with selected devices, including rubber fingers, for prodding animals also will be tested.

At the end of the year work on this project had not progressed to the point that significant results were available.

C. <u>Developing a Physically Integrated Livestock Marketing and Slaughtering Facility</u>

Work on this project had not progressed to the point that significant results were available at the end of the year.

D. Layouts and Work Methods for Hotel Supply Houses

At Stillwater, Okla., a draft of a report entitled "Hotel and Restaurant Meat Purveyors - Custom Service Houses - Improved Methods and Facilities" was tentatively completed and reviewed by operators of custom service houses prior to its submission for clearance for publication. The reviewers made a number of recommendations which should make the report of more value to and more easily understood by the operators of hotel supply houses. At the end of the year the report was being revised to include their suggestions. Progress on a similar study of frozen portion control hotel supply houses includes the tabulation of all field data and the determination of the composition of products to be fabricated by a house handling an assumed annual volume of 3,900,000 pounds.

E. Layouts and Work Methods for Cattle Slaughtering Plants

At Stillwater, Okla., a manuscript entitled "Cattle Killing Floor Systems and Layouts" was completed and submitted for publication. However, the research which is the basis for the report was initiated and data were compiled in cooperation with the Texas Agricultural Experiment Station. The study compares the relative efficiency of the conventional three-bed type, the gravity on-the-rail, and the powered on-the-rail systems. By using the more mechanized on-the-rail systems, a plant annually handling 50,000,000 head of cattle can save as much as \$13,000 a year. The savings in the on-the-rail systems are in the labor costs and are due to smaller crews, improved handling methods and equipment, and less job-regulated wait time. The powered on-the-rail system has the highest production rate per man-hour for dressing line operations -- 2.17 carcasses, as compared with 2.14 for the gravity on-the-rail system, and 1.68 for the three-bed system. An improved layout, based on a killing rate of 24 cattle per hour, has been developed for each system. The layouts show the arrangement of equipment and work areas for an efficient flow of carcasses through the killing floor. A possible method of increasing the killing rate to 35 head per hour without increasing the floor area or disrupting the flow of carcasses is also shown.

COOPERATIVE MARKETING Farmer Cooperative Service

Problem: Farmers continue to expand their use of cooperatives in marketing the products of their farms. In light of the rapid and complex changes taking place in technology and in market organization and practices, research is needed to help farmer cooperatives and other marketing agencies perform needed marketing services both more efficiently and more effectively. Farmer-directors, managers and others, including the public, need more information to assist in making decisions on how cooperatives can maintain and strengthen the bargaining power of farmers, increase efficiency and reduce costs of marketing, and better meet the needs of our mass distribution system for large quantities of products on a specification basis.

Farmer cooperatives are an important part of the distribution system and represent a major potential for meeting farmers' marketing problems in our modern, dynamic system. They are organized and operated to increase farmers' net income. However, cooperatives face many problems in achieving this goal. Cooperatives must find ways to consolidate volume, for example, through internal growth, merger, acquisition or federation, to strengthen their market position and meet the needs of mass merchandising. Ways must be found to reduce costs by increasing efficiency through improved operating methods, better organization and management, and more use of new technologies.

USDA PROGRAM

The Department conducts a continuing long-range program of basic and applied research and technical assistance on problems of marketing farm products cooperatively. Studies are made on the organization, operation and role of farmer cooperatives in marketing. While most of the research is done directly with cooperatives, the results are generally of benefit to other marketing firms. The work is centered in Washington, D. C. Many of the studies, however, are done in cooperation with various State Experiment Stations, Extension Services, and Departments of Agriculture.

The number of Federal professional man-years devoted to research in this area totals 21.2, of which 1.0 man-years are on the cooperative marketing of citrus, 2.7 to cotton, 3.5 to dairy, 1.0 to deciduous fruit, 0.2 to forestry, 1.9 to grain, 2.6 to livestock, 1.3 to oilseeds and peanuts, 1.0 to potatoes, 2.7 to poultry, 0.2 to rice, 1.0 to sheep and wool, 0.1 to sugar, 1.0 to tobacco, and 1.0 to vegetables.

Research also is conducted under contract with land-grant colleges, universities, cooperatives and private research organizations. During the period of this report, contract research was performed by universities and colleges in Florida, Iowa, Louisiana, Montana, North Carolina, North Dakota and Oregon, and by two private research companies.

In addition, 15 case studies of individual or groups of cooperatives were completed. These were concerned with the improvement of operating methods and the feasibility of coordinating the marketing of two or more cooperatives.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

Livestock

1. <u>Pooling and pricing</u>. Additional findings from a study on the efficiency of pooled sales were published in three reports covering feeder cattle, feeder pig, and veal calf pooling operations. Work is continuing on preparation of reports on lamb and slaughter hog pooling operations.

The veal calf publication reported an estimated 154 market agencies in 10 States were pooling calves. They pooled an estimated 668,000 calves in 1959, over 97 percent of which were in Kentucky, Ohio, Tennessee, Virginia, and West Virginia. Other areas of the country seem particularly well suited to the use of pooling, especially the heavy dairying sections. Market agencies estimated that producers received from \$1 to \$2 a hundredweight more for calves that were pooled than they would have received had the calves been sold singly. In addition to this benefit to producers, it was estimated that market agencies saved an average of 48 minutes a sale by selling calves in pooled lots. Assuming an hourly operating cost of \$50, these agencies would have saved an average of \$40 a sale, or over \$2,000 a year. This means a total savings of nearly \$300,000 annually for all agencies engaged in pooling.

Work was completed on a study which showed that, while cooperatives are using USDA grades and paying price differentials for hogs only to a limited extent, these practices appear to be increasing. The study indicated the need for encouraging greater use of grades and price differentials to increase quality production.

2. <u>Livestock integration</u>. More and more cooperatives are looking for ways to integrate their operations to help farmers maintain control over their products further in marketing channels and to become a more effective bargaining force in the marketing of livestock. Integration is taking the form of expanded contract production and marketing pro-

grams and the operation of livestock feedlots and slaughtering and meat processing facilities. Work continues on a study of methods that cooperatives and livestock producer groups can use to successfully integrate their operations.

Three ARA technical assistance studies were initiated to determine the economic feasibility of establishing cooperative livestock feedyards and slaughter facilities in North Dakota, Montana and Oregon. In these studies, being done under contract with the land-grant colleges in these States, four main factors are considered: Supply of livestock, supply of surplus feed grains, kind of markets available, and availability of competent management and labor. Preliminary manuscripts have been prepared by the contractors on those portions of the studies completed to date. Findings from the studies in these three States will be of value to cooperatives and producer groups in other States having similar characteristics when analyzing the feasibility of integrating their operations.

Studies were made on the feasibility of lamb slaughtering plants in the Pacific Northwest and the Middle Atlantic States, and beef plants in the western area.

- 3. Improved market procedures in the Northeast. Work is continuing with the Northeastern States through a new project that seeks to determine the market potential for live animals produced in the Midwest, as well as for beef and hog carcasses and fresh pork cuts now slaughtered and cut in midwestern plants. All type of plants and market outlets will be studied.
- 4. <u>Coordination of marketing</u>. Previous study on the possible advantages of consolidating the operations of cooperatives in California is being continued with analysis of the feasibility of such a move by two associations.
- 5. Improving operating methods. A study made of the operations of a cooperative cattle feedlot in North Dakota alerted management to problem areas and pointed out ways to increase the efficiency of operations and improve member services. A study was completed, in cooperation with the University of Tennessee, on the possibilities for improving cooperative livestock marketing in middle Tennessee. A report suggesting ways cooperatives in the West can improve their services to livestock producers was prepared from a study of the organization, operations, and services of a cooperative in the Intermountain States.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Livestock

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ECONOMICS OF MARKETING Marketing Economics Division, ERS

Problem: Within most agricultural processing industries rapid and drastic changes in their market organization and practices are occurring. These changes are affecting both farmers and consumers. Research is needed to keep abreast of such changes and to indicate their probable consequences. There have been substantial advances in recent years in increasing efficiency and reducing costs through adoption of new technology in producing, assembling, processing, and distributing farm products. However, for producers and marketing firms to remain competitive additional information is needed on margins, costs, economies of scale and efficiencies possible in the marketing of farm products. A significant aspect of the problem in marketing is that this type of information must be obtained from firms engaged in business -- in contrast with other types of research where the problem can be transferred to a laboratory, experimental plot, or other simulated situation. Consequently, it requires the cooperation of people engaged in making their living and assisting with marketing economic research on the side, where their own merchandise, facilities, and opportunity for profit and loss to themselves is involved. Another aspect of the problem is that only large firms can afford this type of research, consequently, public research has been requested for the many smaller firms. Furthermore, there is the need for comparison and analysis where even large firms do not have access to the plants and records of competitors.

USDA PROGRAM

The Department has a continuing program to determine the reason for the changes that are taking place in marketing so that ways can be found to increase the efficiency of the marketing system and make it more responsive to changing public needs. Because more than 50% of the consumer's dollar spent for meat products goes for marketing activities, this work encompasses a wide range of subject matter.

It covers all economic aspects of marketing from the time the products leave the farm until they are purchased by ultimate consumers. Much marketing research is functional in nature and could apply to a number of commodities.

A. Market Potentials for New Products and Uses

This is a continuing long-term program involving agricultural economists, economists and personnel with dual economic and technical training engaged in research to bridge the gap between laboratory developments and commercial adoption so as to assist producers to realize more rapidly and more fully benefits of lowered costs, increased returns, and expanded markets that new products and new uses can afford. Research is carried on in industrial and food uses at Washington, D.C., and five field offices -- agricultural economists are located at each of the four Itilization Research and

Development Divisions - New Orleans, La.; Albany, Calif.; Philadelphia, Pa.; and Peoria, Ill.; and at the Hawaii Agricultural Experiment Station, Honolulu, Hawaii. Of the Federal effort involving 20.5 professional manyears, 4.2 are devoted to animal products.

B. Merchandising and Promotion

This is a continuing long-term program of research in merchandising, management analysis, product distribution, and promotion evaluation, designed to provide useful information to producers, handlers, and distributors by which markets for farm products can be maintained and strengthened.

Merchandising research is conducted to quantitatively measure the impact of selected selling practices and pricing policies on sales of and demand for agricultural products. Research in this area is concerned with specific studies such as: Development of income-expenditure elasticities for selected products; identification of consumer and market profiles; and evaluation of alternative package sizes, displays, pricing techniques, and quality of products on consumer purchases. Along with the merchandising research is a relatively small undertaking involving management type studies designed to improve the efficiency of firms distributing farm products with work at the assembly and wholesale level being emphasized.

Research appraising and analyzing promotional programs of agricultural groups is directed toward studies such as: Organizational structure and procedures of commodity groups for optimum control, coordination, and effective conduct of program; measurement of levels of advertising and promotional intensity necessary to maximize sales; and evaluation of effectiveness of alternative appeals, themes, and techniques in selling farm products. Of the Federal effort involving 17.6 professional man-years, 0.7 are devoted to cross-commodity animal products.

C. Marketing Costs, Margins, and Efficiency

The Department has a continuing long-term program of research in marketing margins, costs, and efficiency designed primarily to provide useful information on the amounts and trends in marketing margins, costs of marketing, labor and equipment requirements, cost standards, economies of scale, and other factors including marketing practices, affecting costs of marketing through all important trade channels and types of firms and for all farm products marketed in commercial volumes. Most of the research is problemsolving in nature, and is conducted by professional agricultural economists. Some studies are conducted in close cooperation with agricultural engineers and members of other disciplines. In nearly all studies close cooperation is maintained with industry and trade groups and with private firms that generously provide essential data and make plant facilities available for observation and the conduct of various market tests. Although most of the research is conducted by personnel in Washington, D. C., a considerable part

of the work is done by USDA professional staff located at field stations in several States. These agricultural economists work closely with State agricultural experiment stations which also share a part of the expense of the cooperative studies.

Of the Federal effort involving 42.2 professional man-years, including cooperative agents paid mainly from Federal funds, 5.8 are devoted to livestock products.

D. Market Structure, Practices, and Competition

The Department has a continuing long-term program of economic research to assist farmers and marketing agencies to adapt to changes in market structure, practices and competition. Work in this area is conducted at Washington, D. C., at field offices in Berkeley, California and Denver, Colorado, at 20 experiment stations under cooperative agreements or contracts, and by a private firm under contract. The Federal scientific effort devoted to economic research in this area totals 42.4 professional man-vears, of which 1.7 is devoted to beef cattle, 4.1 to livestock, and 0.1 to hides.

E. Information, Outlook, and Rural Development

The Department's research program concerning marketing information, outlook, and rural development includes situation and outlook reports concerning prices, costs and margins, employment, marketing services, market structure, means of collecting and disseminating market information, and feasibility of investments in rural areas.

The Department's continuing program of economic research relating to marketing information, outlook, and marketing aspects of rural development is conducted mainly at Washington, D.C.; work on marketing information is conducted at Baton Rouge, Louisiana, Manhattan, Kansas, Columbia, Missouri, Madison, Wisconsin, and University Park, New Mexico; and work on long-term outlook at Berkeley, California, and Corvallis, Oregon.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Market Potentials for New Products and Uses

Leather

Synthetics have been substituted for leather in a number of uses. Market research to appraise the probable extent of this competition and means by which leather may better serve market requirements reveals that leather stands at the crossroads. Shoes, luggage, handbags, and a number of other products, once made chiefly from leather are being made in increasing

quantities from non-leather material. Leather is striking back through technological improvements aimed at lowering cost and improving quality. Developments to date have been helpful, such as glutaraldehyde tanning and brine curing but leather's hope lies in the achievement through research of major technological gains.

B. Merchandising and Promotion

Economics of Pricing, Merchandising, and Labor Utilization in Retailing Meat and Meat Products

This work is being carried out in cooperation with the Ohio State Experiment Station in a sample of retail food stores. A preliminary analysis of data on labor utilization indicated potential savings from more effective planning and coordination of feature promotions, advertising and merchandising, and the use of commodity time requirement data for scheduling and utilizing meat department employees. The impact of features appeared to vary both by type of feature and among stores with the magnitude of the sales variation depending upon the class-of-trade. Preliminary analysis of labor utilization data indicated that at least 90 percent of the variance in man-hours spent processing wholesale cuts into retail cuts is explained by tonnage volume indicating no significant economy of scale. These data along with other data to be collected will be used to develop and test improved managerial techniques that will reduce marketing costs and increase sales of meat products.

C. Marketing Costs, Margins and Efficiency

Livestock

1. Marketing Costs and Margins. A study of accounting records from large independent meatpackers showed about 7 percent of the average retail price of fresh beef went to cover costs to packers for slaughter, shipping and delivery services, compared with farmers' share at about 60 percent.

Allocation of these costs to labor, grading royalties and packaging, procurement and selling transportation and delivery were similar in amount and proportion in January-March 1963, October-December 1962, and the average for January-December 1961.

Retail meat prices appear to adjust to changes in prices of live animals only after lag of five to eight weeks. This lag was observed in the most recent drop in beef prices and appears to be of about the same magnitude as in similar circumstances in past years.

2. Costs of Slaughter. Summary of data from monthly accounting records for $\overline{1962-63}$ for large independent packers showed labor cost about \$1.50 per 100 lbs. of dressed beef; over one-third of packers' total processing

costs of \$4.20 per 100 lbs. Shipping and delivery costs amounted to about \$1.05 per 100 lbs. and fixed overhead to another \$1.00. Local delivery and cost for procurement and selling averaged about \$.35 to .40 per 100 lbs.; transportation to distribution centers about \$.75 per 100 lbs.; packaging, grading and royalties about \$.20 per 100 lbs. Three-fourths of the packers reported costs for grading and royalties.

About two-thirds of labor cost was for slaughter operations, head and offal workup, and the chill cooler; about one-fourth for shipping cooler labor in loading out; and the remainder for hide-cellar operations.

No consistent cost differences could be attributed to size or location of plant, for this sample, but there was a wide range in costs for plants of similar size.

A break-even analysis of changing beef volume showed both that with varying buying prices and selling prices and with costs that vary with changing volume in varying patterns, most packers find that the volume range for profitable operations is narrow and income barely covers costs; attempts to increase profits expanding volume are likely to squeeze operating margins further.

Labor requirements in hog-killing operations ranged from about 1.4 head per man-hour to about 6.3 head per man-hour. Part of the variation is influenced by plant capacity. Most of the variation efficiency among plants seemed to be associated with physical factors such as varying equipment-labor combinations.

- 3. Livestock and Meat Movements in the Southeast. Apparently the movement of hogs in the Southeast is relatively efficient. The hog-pork industry can compete favorably with the Midwest under the current transfer cost structure. However, if hog slaughter costs in the Southeast increase to the level of slaughter in the Midwest, it will be more economical to ship pork to the Southeast than to ship hogs in for slaughter. In this case, the Southeast hog slaughtering industry will be more dependent upon local hog production.
- 4. Costs of Curing Hides. The completed analysis of several hide curing methods indicates that the volume of hides cured in a plant is important in choosing the most efficient method of curing. Pack-salt curing is the lowest-cost method for plants processing fewer than 300 hides a day. The agitated-brine method is slightly more efficient at daily volumes of 300-500 hides, and for larger firms the agitated-brine method definitely is the least costly method. The costs of fleshing were estimated at 16 cents per hide with a volume of 400 hides daily, but only $12\frac{1}{2}$ cents with a volume of 1,000 hides a day.

D. Market Structure, Practices and Competition

Livestock

- 1. Wholesale Marketing Channels. Marketing channels for wholesale meat distribution in the United States have been described and changes in the structure in the wholesale market evaluated on the basis of data from the Census of Manufactures. The decline in the importance of packer branch houses observed since the 1920's appears to be continuing at the same time independent meat wholesalers are becoming more important in the wholesale channels for meats.
- 2. Specification Buying of Meat. Of 1375 chainstore and independent retailers interviewed in Maryland, West Virginia and New Jersey, only 7 reported use of contracts with farmers or livestock feeders. About one-half of the retailers reported specifying grade, Federal or State inspection and sex when purchasing meat; nearly all specified weight range and cut, delivery date, price or pricing basis and method of payment.
- 3. Structure of Texas-Oklahoma Livestock Economy. Except at retail, not many changes in the structure of the Texas-Oklahoma meat industry are evident. However, substantial changes will be required in the near future. Sharp changes in meat retailing and wholesaling have caused changes in procurement practices. Fed livestock are increasingly in demand. As the Texas-Oklahoma livestock-meat economy moves to accommodate the changes in livestock feeding and slaughtering, the number of slaughtering firms will continue to increase, and plants will be larger in size. Fewer wholesaling firms will be required, but those remaining will be larger.
- 4. <u>Structure of Livestock Slaughter</u>. Livestock slaughter has become less concentrated in the hands of the 4 and 8 largest firms. This is true for all species and for amalgamated livestock slaughter. Slaughter plants have also become more specialized. Plant and firm entry and exit vary considerably from year to year, but in each of the past 12 years new capacity has been greater than the capacity withdrawn.
- 5. <u>Pricing Livestock</u>. Annual and semi-annual econometric models of price determination in the beef and pork sectors have been developed and tested. Projections have been made to 1960-1975. Beef production has been disaggregated into fed, nonfed, and dairy components. Interregional least cost of transportation flows for 1955 and 1965 for feeder and slaughter cattle. The daily pricing process of packer buyers and commission firms have been observed at Denison, Iowa, and East St. Louis. Representatives of meatpacking firms have been interviewed regarding daily pricing operations. A panel of 80 livestock producers have been contacted.

Hides, Skins and Leather

Supply and Demand for Leather and Substitutes. The prospective supply of hides resulting from increasing rates of slaughter of domestic livestock will require greatly expanded domestic and foreign markets for hides, leather, and leather goods. Unfortunately, for the hide and leather industries, and indirectly for livestock producers, leather and leather products are experiencing increasingly severe competition from substitute products. If present trends continue, 60 percent of U. S. shoe production may be made of non-leather materials by 1970. Also, by this year, with a decreasing domestic demand for leather, perhaps as much as half of our total hide supply estimated at 34 million hides may have to move into foreign outlets. A major technological breakthrough in tanning appears to offer the greatest promise for making leather more competitive, pricewise, with substitute products. Reducing the costs of curing hides may help some. The value of hides and skins at the packer level is estimated to be about 4 percent of the retail value of leather goods.

E. Information, Outlook, and Rural Development

Leather and Competitive Substitutes, Long-Term Trends in their Supply and Demand

Present trends indicate that 60 percent of the domestic shoe production may use nonleather materials by 1970. With such a decrease in domestic demand for leather, half of our total hide supply, estimated at 33 to 35 million hides, may move into foreign markets by 1970.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Animal Products

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Livestock

- Agnew, Donald B. August 1963. Meatpacker costs and spreads for beef. MTS-150 (Reprinted as ERS-135).
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- Agnew, Donald B. March 1963. Meatpacker costs in fresh beef operations--a pilot survey. Address, meeting of Southwestern division, National Independent Meatpackers Assoc., Dallas, Texas.
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- Fishel, W. L., and Rohdy, D. D. Statistical supplement No. 2 to Southern Cooperative Series Bul. 83. September 1962.
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 - Maki, W. R. November 1962. Cyclical and sernal supply and price patterns for beef. Address at National Beef Confere e.

- Maki, W. R. October 1962. Transportation costs and location of the meatpacking industry. Paper for National Conf. on Regulated and Unregulated Carriers, Transportation Center, Northwestern University.
- Motes, William C. May 1963. Adjustments in retail prices of beef to supply changes. MTS-149. (Reprinted as ERS-123.)
- Northeastern Livestock Marketing Research Committee. March 1963. Specification buying of meats within selected areas of the Northeast. New Jersey Agr. Expt. Sta. Bul. 807.
- Phillips, Victor B. February 1963. Marketing spreads for beef and pork. MTS-148.
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- Wright, N. Gene and Stubblefield, Thomas N. March 1963. Direct sales vs. terminal market for selling fat cattle. Arizona Agr. Expt. Sta. Bul. 152.

Hides, Skins, and Leather

- Thompson, John W. April 1963. Some economic considerations for the hide and leather industry. Speech presented before National Hide Association Training School.
- Thompson, John W. February 1963. Continued increase in edible tallow output anticipated. Reprinted from the Fats and Oils Situation, FOS-216.
- Thompson, John W. February 1963. Appraising the performance of the rendering industry during the last ten years, 1952-61. Speech presented before Minnesota State Renderers Association, St. Paul, Minnesota.

ECONOMIC AND STATISTICAL ANALYSIS Economic and Statistical Analysis Division, ERS

Problem. Because of the instability of the prices he receives and rapidly changing conditions of agricultural production, the farmer stands in special need of accurate appraisals of his economic prospects if he is to plan and carry out his production and marketing activities in an efficient and profitable way. The typical farmer cannot afford to collect and analyze all the statistical and economic information necessary for sound production and marketing decisions. It has long been a goal of the Department to provide the farmer with economic facts and interpretations comparable to those available to business and industry, through a continuous flow of current outlook information; the development of longer range projections of the economic prospects for the principal agricultural commodities; and analyses of the economic implications of existing and proposed programs affecting the principal farm commodities.

USDA PROGRAM

The program of basic research into the <u>factors</u> affecting prices, supply, and consumption of principal agricultural commodities has emphasized four broad research areas: (1) measurement of consumer response to price; (2) measurement of the effect of price and other factors on the production and supply of farm products; (3) measurement of the effect of supply and demand factors on farm prices and prices to consumers; and (4) improvement of statistical techniques for measuring economic relationships.

Changes in emphasis are made from time to time to utilize effectively the professional skills available and to adjust to work having the highest priority. The current emphasis is on a comprehensive analysis of the pricemaking forces in the feed-livestock economy, especially on factors affecting supply. As specific agricultural programs are usually proposed on a commodity basis, the current program is discussed in detail on a commodity basis though much of the actual research is carried on jointly for related commodity groups.

Livestock and Meat. This work involves 1.5 professional man-years located in Washington, D. C. Research on livestock is part of a comprehensive analysis of the price-making forces in the feed-livestock economy. This study gives special attention to the quantitative measures that show what happens to the production of each commodity within the feed-livestock sector following changes in price of one or more of the commodities. The study includes analyses for the United States as a whole and for regions to measure differences in price response and to allow for the important farm and non-farm alternatives available in each region. The present emphasis is on economic factors that affect the supply and price of beef cattle and the demand for feeder cattle and the interrelations among these factors. Results from the beef, hog and feed grain studies along with analyses for milk, eggs, and broilers will be incorporated into an overall analysis of the feed-livestock economy.

The program pertaining to Commodity Situation and Outlook Analysis includes the regular publication of 11 commodity outlook reports; holding of the Annual Outlook Conference in Washington in midNovember; participation of commodity specialists at regional or State outlook meetings or at meetings of farm organizations and agricultural industry groups; preparation and publication of special articles bearing on both the short-run and long-run outlook for farm commodities; issuance of comprehensive statistical bulletins containing the principal economic series pertaining to the various commodities; long-range projections of supply of and demand for the major agricultural commodities; and continuing analysis of the impact of existing and proposed alternative farm programs as they affect output, utilization and prices of these commodities.

The total commodity situation and outlook program currently involves 22 professional man-years.

Livestock and Meat. This work involves 2.5 professional man-years

in Washington and 2.0 professional man-years in Denver, Colorado. outlook and situation program provides a continuing appraisal of the current and prospective economic situation of livestock and meats. These appraisals, developments of interest to the industry, and results of special studies are published 7 times a year in regular issues of the Livestock and Meat Situation, in special additional issues as warranted, quarterly in the Demand and Price Situation and the National Food Situation, and monthly in the Farm Index. A comprehensive analysis of the livestock situation is presented at the Annual Outlook Conference. Outlook appraisals are frequently presented at regional or State outlook meetings, at meetings of farm organizations, and to various agricultural industry groups. Special analyses are prepared from time to time on the probable effect of proposed programs on the price, supply and consumption of livestock and livestock products. Basic statistical series are maintained, improved and published for general use in statistical and economic analysis. A Statistical Handbook, Livestock and Meat Statistics is published annually.

A Western Regional Field Office in Denver, Colorado, conducts a continuing appraisal of the conditions important to the range livestock industry of the West. The results of this activity are published monthly in the Western Livestock Round-Up, and supplemented by special releases and special materials circulated to the Extension Marketing Specialists of the Western Region.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Supply, Demand and Price Analysis

Work on demand for meats included an analysis of the experience in consumption of livestock and livestock products during the last decade as to clues for underlying trends that reflected changes in tastes and preferences. The analysis showed that the demand for beef and broilers shows the most promise in the postwar period. It was found that after allowing for the effects of changes in relative prices and for changes in consumer income, the net increase in per capita demand for beef and broilers was about 1 percent per year. On the other hand the trend in per capita demand for pork, exclusive of changes arising from price and income changes, was downward at a rate of 1 percent per year.

A technical bulletin was issued in December 1962 which describes the major economic relationships that affect the supply and price of hogs. This bulletin won first prize in the American Farm Economic Association Awards for published research competition. The bulletin contains an explanation of the factors involved in the cyclical behavior of production and prices of hogs, and measures the effect of various factors such as the prices of feed, beef and poultry, and consumer income on the hog economy. Results of this study were reported in detail in last year's report.

Work on price and supply of beef cattle during the current reporting period was centered on an analysis of economic factors that influence the inventory accumulation and depletion of beef cattle. The inventory of beef cattle is depicted as being made up of the number of (1) beef cows on farms (2) beef cattle on farms 1 to 2 years old (3) beef calves on farms and (4) beef bulls Supply and demand relations are being formulated for each component part and preliminary statistical results have been obtained for some of the relationships. Research during the year has been mostly on items (1) and (2). The demand for beef cows for slaughter has been related to (1) price of utility slaughter cows, (2) price of heifers and (3) price of slaughter hogs. The supply of beef cows for slaughter has been related to (1) price of utility slaughter cows, (2) price of slaughter calves, (3) number of beef heifers brought into beef herd, and (4) range conditions. The demand for heifers to be brought into herd was related to (1) price of heifers, (2) the number of beef cows slaughtered, and (3) price of calves. On the other hand the supply of heifers to be brought into beef herds was depicted as depending on (1) price of heifers, (2) beef-corn ratio, and (3) the number of calves on farms the previous year.

As part of the cattle study, regional locational patterns of different classes of cattle have been made. The individual regional influences of the changing location patterns are being traced and related to the United States as a whole.

B. Situation and Outlook Analysis

The increase in beef production this year exceeded the increase in demand, and fed cattle prices fell. While part of this drop in the opening months of 1963 was recovered by mid-year, prices of fed cattle remained well under 1962 levels for the second half of the year. Continued large production is expected to cause some further weakening of cattle prices in the first half of 1964. Pork production also increased in 1963 and pork prices averaged lower than a year earlier. Pork supplies in 1964 likely will be much the same as in 1963 and prices are expected to average slightly higher, especially during the winter and early spring months. Liquidation of sheep and lambs, which began in 1960, continued the past year although the rate slowed somewhat. Lamb prices in the first quarter of 1963 were much higher than a year earlier, but in the remainder of the year averaged about the same as those in 1962. Iamb and mutton production in 1964 is expected to be only a little smaller than a year earlier and 1964 may mark the low point in the present downswing. Prices may be about the same as in 1963.

A special situation report on livestock was released in April to help producers, marketers, and consumers to understand and adjust to changes in the economic situation brought about by the sharp decline in fed cattle and hog prices. It presented basic information on the prevailing fed cattle and barrow and gilt price situation and discussed factors that were likely to influence the future.

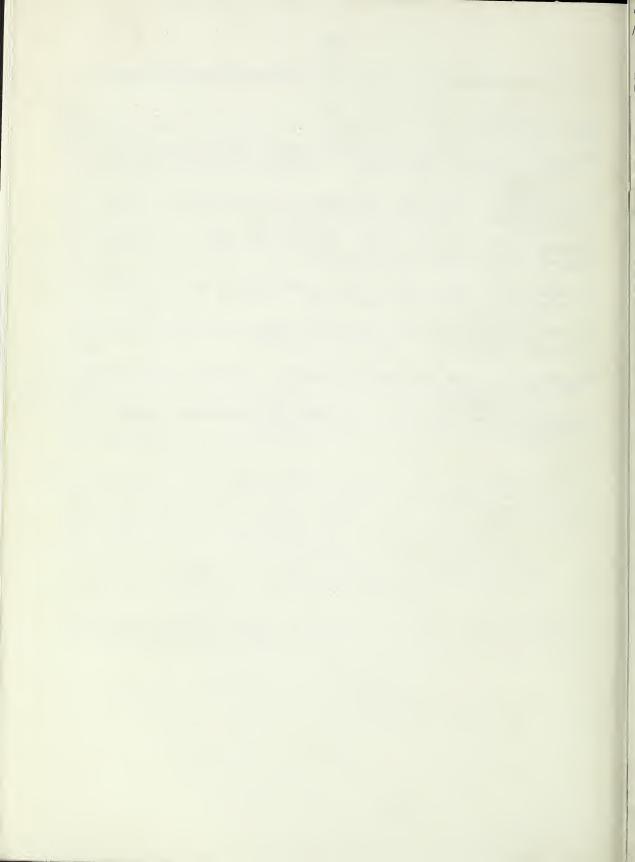
To gauge the probable future trend in consumption, special analyses were made of the uptrend in beef and the downtrend in pork in the last decade. Emphasis was also given to study of the cattle cycle which began its current upswing in 1959. Long-run projections (5 years) were developed for cattle, hogs, and lambs as part of a set of ERS projections for the farm economy as a whole. Work on seasonal patterns of prices and production for various classes and grades of livestock and livestock products was continued. Other work in progress includes an analysis of the regional distribution of livestock production.

<u>Livestock</u> and <u>Meat</u> <u>Statistics</u>, Statistical Bulletin No. 333, the first master issue since the original comprehensive Statistical Bulletin No. 230, was released in July 1963.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Livestock and Meat

- Gerrity, Martin V. August 1963. Common market regulations and U.S. livestock and meat product exports. Livestock and Meat Situation.
- Motes, William C. July 1963. Adjustments in retail prices of beef to supply changes. Livestock and Meat Situation.
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A Summary of Current Program and Preliminary Report of Progress

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BEEF CATTLE RESEARCH

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United States Department of Agriculture and related work of the State Agricultural Experiment Stations

CURRENT SENIAL RECORDS

This progress report is primarily a research tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of research progress include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued during the past year. Current agricultural research findings are also published in the monthly U.S.D.A. publications, Agricultural Research and The Farm Index.

UNITED STATES DEPARTMENT OF AGRICULTURE Washington, D. C. 20250

December 1, 1964



ADVISORY COMMITTEES

The research program of the Department of Agriculture is reviewed annually by the following advisory committees:

- 1. Farm Resources and Facilities Research
- 2. Utilization Research and Development
- 3. Human Nutrition and Consumer Use Research
- 4. Marketing Research
- 5. Agricultural Economics Research
- 6. Forestry Research
- 7. Animal and Animal Products Research
- 8. Cotton Research
- 9. Grain and Forage Crops Research
- 10. Horticultural Crops Research
- 11. Oilseed, Peanut and Sugar Crops Research
- 12. Plant Science and Entomology
- 13. Tobacco Research

ORGANIZATIONAL UNIT PROGRESS REPORTS

The source materials used by the advisory committees are of two types. First, there are Organizational Unit Reports that cover the work of the Divisions or Services listed below. The number prefixes refer to advisory committees listed above that review all of the work of the respective Divisions or Services.

Agricultural Research Service, (ARS) Economic Research Service, (ERS)

- 1 Agricultural Engineering
- 1 Soil & Water Conservation
- 2 Utilization -- Eastern
- 2 Utilization -- Northern
- 2 Utilization -- Southern
- 2 Utilization -- Western
- 3 Human Nutrition
- 3 Clothing and Housing
- 3 Consumer & Food Economics
- 4 Market Quality
- 4 Transportation & Facilities
- 7 Animal Husbandry
- 7 Animal Disease & Parasite
- 12 Crops
- 12 Entomology

- 4,5 Marketing Economics
- 4 Farm Production Economics
- 5 Resource Development Economics
- 5 Economic & Statistical Analysis
- 5 Foreign Development & Trade
 - Analysis
- 5 Foreign Analysis

Other Services

- 4,5 Farmer Cooperative Service (FCS)
- 4,5 Statistical Reporting
 Service (SRS)
- 6 Forest Service (FS)

SUBJECT MATTER PROGRESS REPORTS

The other type of report brings together the U.S.D.A. program and progress for the following commodities or subjects:

3 - Rural Dwellings 6 - Forestry (other than Forest Service) 7 - Beef Cattle

7 - Dairy 7 - Poultry 7 - Sheep and Wool 7 - Swine

7 - Cross Specie and Miscellaneous 11 - Sugar Animal Research

8 - Cotton and Cottonseed 9 - Grain and Forage Crops

10 - Citrus and Subtropical Fruit 10 - Deciduous Fruit and Tree Nut

10 - Potato 10 - Vegetable

10 - Florist, Nursery & Shade Tree

11 - Oilseeds and Peanut

13 - Tobacco

A copy of any of the reports may be requested from Max Hinds, Executive Secretary, Animal and Animal Products Research Advisory Committee, Research Program Development and Evaluation Staff, U. S. Department of Agriculture, Washington, D. C.

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INTRODUCTION

This report on beef research covers work directly related to the production, processing, distribution, and consumption of beef cattle and resulting products. The information has been assembled from the organizational unit reports of the several divisions. This report does not include extensive cross-commodity work, much of which is basic in character, which contributes to the solution of not only beef problems but also to the problems of other commodities. Progress on cross-commodity work is found in the reports of the several divisions such as Soil and Water Conservation, Human Nutrition, Transportation and Facilities, Farm Production Economics, Foreign Development and Trade Analysis, and Cross-Specie and Miscellaneous Animal Research.

This report is devoted to the 17 "problem areas" shown in the table of contents. For each area there is a statement of (1) the <u>Problem</u>, (2) the USDA and Cooperative Program, (3) Program of State Experiment Stations, (4) a summary of <u>Progress</u> during the past year on USDA and cooperative work, and (5) a <u>list of Publications</u> resulting from USDA and cooperative work.

Beef research can be divided into three major categories, i.e., that supported by (1) Federal funds appropriated to the research agencies of the United States Department of Agriculture, (2) Federal and State funds appropriated to the 53 State Agricultural Experiment Stations, and (3) private funds allotted, largely by the beef industry, to research carried on in private laboratories or to support of State Station or USDA work. For all three categories it is estimated that about 1,300 scientists are engaged in research dealing specifically with the production, processing, distribution, and consumption of beef and its products. Support of their work involves an annual expenditure of between 30 and 35 million dollars. This amounts to 0.6% of the cash farm receipts from the sale of beef cattle and 0.4% of the retail cost of beef. Of the 1300 scientists engaged in beef research, approximately 19% are employed by the Department of Agriculture, 20% by the State Experiment Stations, and 61% by other universities, foundations, and private industry.

Research by USDA

Farm research pertaining to beef is conducted in the Agricultural Research Service divisions of Agricultural Engineering, Animal Disease and Parasite, Animal Husbandry, and Entomology. The work comprises investigations of breeding, physiology, nutrition, diseases, insects, housing and management, involving 154 professional man-years of scientific effort. This includes research on cattle diseases and parasites that is applicable also to dairy of which 58 man-years are devoted to domestic diseases, 22 to parasites, and 28 to foot-and-mouth disease.

Nutrition, consumer, and utilization research pertaining to beef is conducted in the Agricultural Research Service divisions of Human Nutrition, Consumer and Food Economics, and Eastern Utilization. The work comprises iv

investigations of composition and nutritive value; physiological availability of nutrients and their effects; new and improved methods of preparation, preservation, and care in homes, eating establishments and institutions; and with the processing phase involving slaughtering the animals and processing the meat, tallow and hides. Also, it is concerned with improved equipment and processes. The work in these divisions involves all professional man-years of scientific effort.

The utilization research in meat processing and products, animal fats and oils, and hides, skins, and leather which involves more than one species - and if done with one specie may be applicable to others - is discussed in "Cross Specie and Miscellaneous Animal Research" progress report.

Marketing and economic research pertaining to beef is carried on within four Services: Agricultural Research Service, Economic Research Service, Farmer Cooperative Service, and Statistical Reporting Service. The work comprises (1) physical and biological aspects of assembly, packaging, transporting, storing and distribution; (2) economic aspects of marketing costs, margins and efficiency, market potential, supply and demand, and situation and outlook; (3) cooperative marketing; and (4) consumer acceptance studies. The divisions in which the work is conducted are: Market Quality, ARS; Transportation and Facilities, ARS; Marketing Economics, ERS; Economic and Statistical Analysis, ERS; Marketing Division, FCS; Standards and Research, SRS. The scientific effort involved by these divisions amounts to 20 professional man-years.

Interrelationships among Department, State and Private Research

A large part of the Department's research is cooperative with State Experiment Stations. Many Department employees are located at State Stations and use laboratory and office space close to or furnished by the Station. Cooperative work is jointly planned, frequently with the participation of representatives of the producers or industry affected. The nature of cooperation varies with each study. It is developed so as to fully utilize the personnel and other resources of the cooperators which frequently includes resources contributed by the interested producers or industry.

Including both cooperative and State Station projects beef research is carried on in 47 of the 53 State Experiment Stations. The types of work to which the largest amount of effort is devoted includes breeding, physiology, nutrition and management, diseases and parasites, marketing economics, and utilization research on meat and animal fats. There is regular exchange of information between Station and Department scientists to assure that the programs complement each other and to eliminate unnecessary duplication.

Privately supported beef research emphasizes the solution of scientific production, processing, and marketing problems. Much of it utilizes the results of basic work done by State Station and Department scientists.

About 1/3 of industry's contribution to the research effort pertains to farm research. In contrast with the poultry industry where practically all breeding research is done by industry, very little is done by industry in beef, except the work of large firms like the King Ranch which developed the Santa Gertrudis breed. The cope of operation required for a successful research program in breeding beef cattle, because of the size of animal and length of life cycle which tie up a substantial amount of funds, is undoubtedly a factor contributing to the amount of public research.

Another one-third of the research effort is in the utilization field. In contrast with the public research in basic work the industry program places strong emphasis on developmental activities and solving of immediate problems. The work of meatpackers is devoted to finding industrial utilization of by-products, quality control devices, improved formulation of products, improved handling and plant arrangement. Independent laboratories and foundations take on short time problem-solving for clients in the meat industry. Pharmaceutical firms carry on research on extraction of biologically active substances from meat by-products such as hormones from glands, and with the development of agents, such as antibiotics for use in meat processing.

The contributions of beef producers and industry to the work of the State Stations and the Department have been an important factor in the success of their research programs. Producers offer herds and facilities for testing products and practices used in production. Likewise, processors and retailers offer facilities and products for use by public research agencies. Many problems in the economics of marketing cannot be transferred to a laboratory, experimental plot, or other simulated situation. The results of economic research conducted cooperatively is of great value to industry, especially in cases where public research can provide comparison and analysis. Even large firms that have a research staff do not have access to the plants and records of competitors.

Examples of Recent Research Accomplishments by USDA and Cooperating Scientists

Urinary calculi reduced by ammonium chloride. A practical application of previous research is the increased use by both ranchers and feedlot operators of ammonium chloride as a control measure in the prevention of urinary calculi. It is now in use in several western and southwestern States. Within recent months there has been a large increase in requests for information from individuals and feed manufacturers for details on the use of ammonium chloride in feeds and feed additive mixtures. This is a good example of basic information being applied to beef cattle production in a relatively short time.

- Heterosis found in crosses of beef breeds. Systematic study of straightbred animals of the Angus, Hereford, and Shorthorn beef breeds as compared to all possible crosses among them indicate heterosis in pre- and postnatal calf survival rates and in weaning weight of individual calves large enough that pounds of calf weaned per cow bred is at least 10% greater in crossbred as compared to straightbred matings. Heterosis of a lower order of magnitude is found in postweaning growth but not in carcass characters. Preliminary results suggest rather important heterotic effects on brood cow performance. These results may have important implications for commercial beef production in the United States.
- Bovine Vibriosis. Researchers at the National Animal Disease Laboratory, reported that all heifers bred to an infected bull became infected at the first service. Only 2 of 12 infected heifers became pregnant as compared to 6 pregnancies in the 7 noninfected control heifers. The presence of a moderate inflammatory process in the uteri of the infected heifers suggested the underlying cause for the lack of conception.
- Biochemical Effects of Agricultural Chemicals. Research at the Division's Toxicological Research Laboratory at Kerrville, Texas, has shown that the feeding if vitamin A to cattle increased their susceptibility to poisoning by one of the systemic insecticides. This was particularly evident when cattle were also given phenothiazine drenches for internal parasite control. Four important enzyme systems were affected by the vitamin A-phenothiazine-systemic insecticide combination. In another experiment, it was found that Brahman cattle were more susceptible to poisoning from two insecticides than were European breeds. This kind of research is helping to provide a fuller understanding of some of our pesticide problems in livestock.
- Reproductive Diseases: Much progress is being made in determining causes of reproductive diseases in cattle. California, Colorado and Ohio workers recently have discovered that outbreaks of abortion can be caused by infectious bovine rhinotracheitis a virus disease which previously was thought to affect only the respiratory tract of cattle. Connecticut research has found some cases of bovine sterility to be due to infection with Mycoplasma an organism with bacterial and viral-like characteristics. Severe outbreaks of mastitis also were traced by these workers to a similar Mycoplasma agent.
- Ventilation of Livestock Buildings: Research in cooperation with State

 Experiment Stations has obtained much needed basic data on the heat
 and moisture given off by cattle, hogs, and poultry, and on the
 influence of building environment on production and feed consumption.
 The heat and moisture dissipation data are considered basic design
 data for ventilation systems of poultry, dairy, and swine buildings.

They appear in design handbooks, including the 1964 Guide and Data Book of the American Society of Heating, Refrigeration, Ventilating, and Air Conditioning Engineers, and are used by makers of ventilating equipment, prefabricated buildings and package buildings as well as by specialists advising farmers on their own construction. Building improvements resulting from the above research have contributed to the substantial rise in efficiency of livestock production that has occurred during the past decade.

Improved Data on Composition of Beef: The nutrient composition of beef has been intensively investigated in order to provide more complete and accurate data for tables of food composition. A method has been developed for establishing consistent relationships among carcasses of different grades and among cuts prepared from a carcass. This study provides for the first time average data (1) for carcass beef at the six USDA grades--Prime, Choice, Good, Standard, Commercial, and Utility; (2) for all the principal retail cuts of the three major grades--Choice, Good, Standard; and (3) for cooked meat prepared from these cuts. The procedures developed for this study will be useful guides to research workers in investigations on other kinds of meat.

New Facilities for Handling Meat and Poultry in New York City: As a result of studies by the Transportation and Facilities Research Division, ARS, an additional \$40.0 million complex of facilities is being planned for handling meat and poultry, for which the New York City Board of Estimate has allotted \$6.1 million for site acquisition and design. The facilities are being planned adjacent to the new \$36.0 million fruit and vegetable facility that is under construction at Hunts Point and will replace the 14th Street and Brook Avenue Markets. Total annual saving in handling fruits, vegetables, meat, and poultry in new facilities is estimated to be almost \$25 million.

Biodegradable Detergents from Tallow: Recent investigations by Eastern Utilization Research Laboratory Chemists indicate that detergents prepared from tallow are biodegradable. Included among the tallowbased detergents tested were the alpha-sulfo acids and their esters and tallow alcohol sulfates and their modifications. Activated sludge digestion tests have shown these to be more easily biodegradable than either branched-chain or linear alkyl benzenesulfonates (ABS and LAS). From companion Division research, it is known that alpha-sulfo acids prepared from tallow are effective lime soap dispersing agents. For these reasons, such compounds may prove useful in soap-detergent combinations. This research also has shown that certain esters of the alpha-sulfo acids are especially effective as wetting agents. Since they compare in effectiveness with the best known commercial wetting agents and can be made very cheaply, commercial interest in them seems assured. Manufacture of alpha-sulfo acids and their esters is being undertaken by several commercial concerns. In the meantime, research is being done to develop more accurate means for evaluating biodegradability necessary as guides for development of detergents that are more viii easily biodegraded.

Price Spreads for Beef: The necessity for having readily available information on prices and price spreads at all levels of the marketing process is well illustrated by recent developments in the beef market. The existence of this information over a period of years made is possible for the Economic Research Service to report and analyze current developments in prices and margins for beef during a period of marked public interest.

There is a long-time upward trend in the margins for beef, similar to that for most food products but at a more rapid rate. In addition, there is a short-term cyclical movement of margins in response to changes in farm prices. Changes in retail prices tend to lag behind those in farm prices--in general, about the length of time it takes for beef to move through marketing channels. Retail prices tend to move more slowly than farm prices both upward and downward.

The biggest part of the increase in a farm-to-retail spread has been at the retail level. Retail margins have risen faster for beef than for other meats or for all foods. Apparently, retailers have responded to the surging demand for beef by shifting a part of the overhead of the store operation from other products to beef.

Beef Imports: Because of growing beef imports in 1963 and price pressures in the domestic cattle market, a special analysis was made to measure the relative importance of domestic fed beef production, cow beef production, beef imports, supplies of competing meats, and other factors on fed cattle and cow prices. The analysis indicated that a one-pound per capita change in fed beef production resulted in a change in the opposite direction of 50 cents in the Choice steer price at Chicago; a one-pound change in the aggregate of cow beef production and beef imports changed the Choice steer price by about 30 cents in the opposite direction; and most of the variation in fed cattle prices in the past two years was explained by variation in domestic steer and heifer beef production. Results from the study are useful in putting imports in proper perspective, in making price forecasts, and in appraising the effect of the government beef purchase program on cattle prices.



I. FARM RESEARCH

BEEF CATTLE - BREEDING Animal Husbandry Research Division, ARS

Problem. Expression of each of the productive and carcass traits of beef cattle varies from breed to breed and between animals within each breed. The beef cattle producer is constantly striving to achieve excellence in one or more of these traits. Frequently his failure to choose the best animals for breeding stock for the most effective mating program results in less than maximum progress. Often the beef cattle producer does not know how to identify, evaluate, and utilize the existing variability to achieve his aim. Research information is needed on heritability of economic traits in beef cattle, genetic and phenotypic correlation between these traits, effectiveness of various selection and breeding programs, and assessment of traits most useful in beef cattle improvement.

USDA AND COOPERATIVE PROGRAM

The beef cattle breeding research in the United States has developed as a coordinated program of the USDA and the State experiment stations. It is a continuing program of both applied and basic research carried on by geneticists, animal physiologists, and animal husbandmen. Early efforts in the improvement of beef cattle through performance testing were made by the USDA at Miles City, Montana, and Beltsville, Maryland. With the advent of regional research, efforts by the State stations were greatly increased and the individual programs were coordinated through regional research projects in three of the important beef cattle producing regions. This joint activity has been and remains characteristic of beef cattle breeding research, and the resulting program is an integrated effort combining to the best advantage the resources of the State experiment stations and the USDA.

The regional project in the South is S-10, Improvement of Beef Cattle for the Southern Region through Breeding Methods. Much of this region is subtropical in climate and in many cases cattle used in other areas are poorly adapted. Environmental conditions adversely affecting survival, reproductive regularity and growth are encountered. Research includes projects at 13 State stations and at the USDA stations at Jeanerette, Louisiana; Front Royal, Virginia; and Brooksville, Florida.

In the Western region the beef industry is largely geared to range conditions with many cattle shipped to areas of abundant grain supply for fattening. Ability to make maximum use of forage available on the range is an important consideration. These problems are studied through regional project W-1, The Improvement of Beef Cattle through the Application of Breeding Methods. Research includes projects at 12 State stations and at the USDA station at Miles City, Montana.

Similarly, NC-1, Improvement of Beef Cattle through Breeding Methods, is geared to problems of the beef industry in the North Central region where

beef is produced on farms with pastures of high productivity and ample grain supplies for feedlot finishing. Research includes projects at 12 State stations and at the USDA stations at Fort Robinson, Nebraska, and Fort Reno, Oklahoma.

The Federal scientific effort devoted to research in this area totals 16.1 professional man years. Of this number, 1.3 are devoted to performance testing, 4.4 to genetics and interrelations of performance traits, 1.2 to genetic-environmental interactions, 6.2 to selection and systems of breeding, and 3.0 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

To a greater degree than many other research programs, beef cattle breeding research has developed as a coordinated program of the USDA and the State agricultural experiment stations. This has been achieved to a large degree by cooperative research activities under three regional beef cattle breeding projects. This coordinated program is described amply by the material appearing under the USDA and Cooperative Program. The several examples cited describe research of a cooperative nature, much of it conducted at the State experiment station locations. The reader is referred to this section of the Division report with these comments, and no attempt will be made to summarize the State programs separately.

The total research effort on beef cattle breeding research by the State agricultural experiment stations is 40.5 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

As compared to other disciplines, knowledge accumulates slowly in Beef Cattle Breeding and Genetics due to (1) low reproductive rates, and (2) high maintenance and operational costs per animal; i.e., per genetic unit. In the material which follows an attempt has been made to include only items on which specific analyses were completed or summaries made during the year and to relate these to studies at other locations or in earlier years many of which have already been covered in progress reports of previous years.

A. Selection and Systems of Breeding

1. Effectiveness of selection for economically important traits. The estimates of genetic parameters; i.e., heritabilities and genetic correlations, give us a basis for projecting what genetic progress should be possible. However, only actual changes resulting from selection can be considered conclusive in determining progress actually possible. Most beef cattle breeding research herds have not been in existence for enough years or generations to permit good estimates of responses to selection. An exception to this generalization is the line 1 of Hereford cattle maintained at Miles City, Montana, under selection for growth and body conformation since its foundation in 1934.

An intensive study of amount and apparent effectiveness of selection in this line was completed during the year and, after allowing for trends in environmental levels, indicates genetic changes of +9.7 lb. in birth weight, +30.0 lb. in weaning weight and +6.5 points in weaning conformation score over a 25 year period.

The data did not permit accurate estimates of environmental time trends for postweaning performance traits. Gross or phenotypic time trends were positive and per generation amounted to 15 lb. in 196-day postweaning gain of bulls, 32 lb. in 18-month weight of heifers and 1.6 points in 18-month conformation score. Indications are that substantial portions of these changes are genetic, but exact estimates of the genetic portion are not possible. No intentional selection was made for mature cow weight but it increased substantially during the period under study.

This study strongly suggests that, for growth and conformation traits, response to selection was approximately as great as would be expected from previously calculated genetic parameters. (AH d1-2)

Another study on closed lines of Hereford cattle at Havre, Montana, indicated positive genetic trends for birth and weaning weights. (AH dl-17)

In a Nevada study, calves in lines selected for several years for rate of gain and economy of gain were 40 lbs. heavier at the end of the test period than calves from a line selected for conformation. Bulls from the rate of gain and economy of gain lines produced larger carcasses with greater rib area than carcasses from bulls in the conformation line. (AH d1-36)

Data from an Oregon study on selection techniques indicated that more progress could be made by testing and selecting animals in the environment under which the animals are expected to produce. (AH d1-19)

2. <u>Crossbreeding</u>. Data accumulating from several crossbreeding experiments continue to indicate important heterosis effects on several traits.

A comprehensive summary of the heterosis effects on preweaning traits was made at the completion of the first phase of the crossbreeding experiment at Fort Robinson, Nebraska. This study includes the Hereford, Angus, and Shorthorn breeds and all possible crosses among them. A total of 751 calves were involved with approximately equal numbers of crossbreds and straightbreds.

The summary showed a significant heterosis effect on birth weight, average daily gain from birth to weaning, weaning weight, and weaning conformation score. The heterosis effect on weaning weight was $19.4~\mathrm{lbs}$. The heterosis effect on weaning weight in the Hereford x Angus and reciprocal and Hereford x Shorthorn and reciprocal crosses was approximately one and three-fourths

times as great as in the Angus x Shorthorn and reciprocal cross. The analysis revealed that the heterosis observed could be attributed to breeds rather than to sires within breeds.

Additional data were collected during the year on the heterosis effects on postweaning growth and carcass characteristics. These results continue to reveal a heterosis effect for postweaning growth rate in both steers and heifers and a heterosis effect on feed efficiency in steers. Individual feed consumption has not been measured in the heifers. When slaughtered at the same age but at slightly heavier weights crossbred steers have had slightly fatter carcasses than the straightbreds.

Results continued to show a slight advantage for the crossbreds over the straightbreds in post-natal mortality. Crossbred females continue to reach puberty at younger ages. Crossbred females have required fewer services per conception, have conceived earlier in the breeding season with a higher percentage conceiving during a three-months breeding season. Crossbred cows have weaned calves approximately four percent heavier than straightbred cows with both groups of cows nursing crossbred calves. (AH d1-12)

A summary of five years of data at the Alabama station with British breeds indicated that crossbred steers had an advantage of 40 pounds in adjusted weaning weight over purebred steers, a 0.15 pound advantage in average daily gain up to weaning, a 70 pound advantage in chilled carcass weight, and slight advantages in grade over the purebred steers. When finished in a uniform feeding period, the crossbred steers were fatter (0.12 of an inch greater fat thickness over the rib eye), but there was no difference in meatiness or tenderness, as measured by rib eye per hundred pounds carcass and Warner-Bratzler shear, respectively. The crossbred heifers weaned heavier than purebred heifers. However, there were no differences in postweaning performance between the two breeding groups. Two years' data with limited numbers suggests that crossbred dams are superior as mother cows. (AH d1-29)

Additional information from two stations on subsequent crosses after the single cross continues to indicate the advantages of crossbreeding schemes over straight-breeding systems. Three years' data from the Louisiana Station, involving single crosses, backcrosses, three-breed crosses, and straightbreds, indicate that the single crosses are slightly superior to backcrosses and three-breed crosses in rate of gain on feed, but the backcrosses and three-breed crosses excel the single crosses in adjusted weaning weight at 205 days. This indicated the advantage of the crossbred dam in calf production. The average weaning weight at 205 days for 93 straightbred calves involving Angus, Brahman, Brangus, and Hereford, was 390 pounds, as compared to 155 single cross calves averaging 418 pounds, 195 backcross calves averaging 459 pounds, and 194 three-breed cross calves averaging 463 pounds. The heavier weaning weights of crossbred calves, coupled with the higher percentage calf crop, give crossbred cows a substantial advantage over purebred cows in pounds of calf weaned per cow bred.

The Louisiana data are supported by work at the Virginia Station where calves from crossbred dams had a higher daily gain to weaning. This study is designed to breed purebred dams with crossbred bulls and crossbred dams with purebred bulls, so that all calves are, thus, either three-breed or back-crosses in all possible combinations of the three breeds - Angus, Hereford, and Shorthorn - involved. This permits an estimate of heterosis in the dams without confounding with heterosis in the calves. In one year's data from the Virginia Station, the crossbred dams had a calving percentage born of 97 percent, as compared to 92 percent for the purebred dams, but both the purebred and crossbred dams weaned 88 percent calf crops. (AH d1-7)

Studies in progress in Ohio and at Miles City, Montana, as well as the Louisiana study, indicate rapid rates of gain for the Charolais and heterosis in both pre- and postweaning gain for crosses of this breed with British breeds.

Crosses of Charolais and Brown Swiss with British breeds have consistently resulted in faster growth than in the British breeds. (AH dl-1, AH dl-10)

In addition to evaluation of total fertility, studies are underway at several locations on heterosis effects on some of the traits of apparent low heritability contributing to or related to the total reproductive process. Age at puberty has been reduced in crossbreds as compared with averages of the parental breeds. The crossbred-straightbred difference is greater at low levels of nutrition. Weight differences are not as great as age differences in puberty leading to the conclusion that the earlier attainment of puberty by crossbred heifers is at least in part accounted for by more rapid growth rates. Breed differences in age at puberty are large with Zebu types being older. This tendency is also transmitted in crosses. (AH d1-30, AH d1-33, AH d1-37)

At Miles City, Montana, attainment of puberty by crossbred and purebred bulls is being studied with the criteria being ages first ejaculation, production of sperm, production of motile sperm and production of sperm in adequate numbers for classification. In all criteria the crossbreds have been younger than straightbreds. (AH d1-33)

In the limited number of observations yet made, the interval from parturition to first estrus has been slightly but probably not significantly shorter in crossbred cows as compared to averages of the parental breeds. Conception rate at first service has been markedly better. (AH d1-30, AH d1-37)

3. <u>Inbreeding and linecrossing</u>. Inbreeding studies are of a long-time nature and the results of the two summaries discussed below must be considered as progress reports. In the mildly inbred line 1 Herefords at Miles City, Montana, increased inbreeding had a detrimental effect on all traits studied. The effect of inbreeding on weight reached a peak at 18 months of age in heifers and declined somewhat at mature weights. Final weight off

test (12-13 mo.) was affected more by increased inbreeding than weights taken earlier in life in bulls.

Increases in inbreeding of dam had a detrimental effect on growth from birth to weaning and weaning weight, presumably through decreased milk production. This effect was completely compensated for at 18-month weight in heifers and was greatly reduced in bulls for final weight off test (12-13 mo.).

There was a differential response by sex to inbreeding of calf and inbreeding of dam in weanling traits. Inbreeding of calf had a more pronounced effect on females than on males, whereas inbreeding of dam had a greater effect on preweaning gain, weaning weight, and weaning score of bulls than on heifers. It is postulated that bulls, having a greater growth potential, are held back more than heifers by the decreased milk supply of their dams that is associated with increased inbreeding of dam. This maternal environment may mask the response to inbreeding of calf in bull calves to a greater extent than in heifers.

In the selection study utilizing these same data discussed in an earlier section, a tendency was found to select replacement breeding animals with lower than average levels of inbreeding. Since no intentional or direct selection was made for less highly inbred animals, the tendency to select less inbred animals was due to their higher performance. It is also likely that selected individuals may be less homozygous than their calculated inbreeding coefficients would indicate. (AH d1-2)

Data from the inbreeding study at the Front Royal, Virginia, Station, where "type" and "growth" data on 2440 calves from birth to weaning were analyzed, indicate large differences among lines in response to inbreeding. This is shown by the difference between foundation and inbred lines for birth and mid-summer weights, gain to weaning, and weaning type score. Weights and gains of the calves from the "growth" herd exceeded those of the "type" calves, and, conversely, "type" calves had higher conformation than did "growth" calves. Response to inbreeding of calf and of dam was nearly opposite in Angus and Shorthorn calves. For example, in Angus each additional 1 percent inbreeding of the calf decreased average daily gain to weaning by -.0056 pounds; whereas, in Shorthorns, the value was -.0031 pounds. In contrast, similar regressions on percent inbreeding of the dam were -.0012 pounds for Angus and -.0047 pounds for Shorthorns. (AH d1-4)

Results to date from crossing inbred lines show heterosis as compared to line performance and strongly suggest fairly important specific combining ability for some traits with results from year to year being consistent. (AH d1-2, AH d1-16)

B. Performance Testing

Attention to improving methods for evaluating performance in beef cattle is continuous in most projects. The most significant overall recent trends are

increased attention to (1) carcass evaluation, including methods of estimating carcass characteristics from live animals, and (2) evaluation of fertility and the components or factors upon which it depends. Routine evaluations of these traits will make more comprehensive future genetic analyses possible.

1. Carcass traits. Genetic improvement of carcass traits must be based on progeny tests and is bound to be slow and expensive unless or until ways of better estimating potential carcass characteristics of live animals can be developed. For this reason, this problem is receiving major emphasis.

The Thermistor Thermometer is a highly sensitive instrument which, when inserted through a small slit in the skin, measures differences in temperature of fat and lean. This permits estimates of fat thickness. In two studies correlations of +.5 and +.62 were observed between estimated live fat thickness and carcass fat thickness at the 12th rib. (AH d1-31,AH d1-12)

Ultrasonic devices permit estimates of fat thickness and depth of muscles through measurement of reflected ultra-high frequency sound waves from tissue interfaces. A number of studies show correlations of +.6 to +.8 between live estimates of fat thickness and rib eye area and carcass measurements. There is evidence of operator and machine differences. (AH d1-8, AH d1-9,AH d1-12)

A Utah study indicated promise for a simple probe device as a means of estimating fat thickness in live animals and a high correlation between its estimates and those of a new ultrasonic device. (AH d1-20)

In a comprehensive study involving subjective evaluation of certain carcass characteristics in live cattle, the correlations between live estimated fat thickness and fat thickness measured in the carcass were in the .5 to .6 range. The estimates involving actual percent of trimmed, boneless retail cuts from the round, loin, rib and chuck showed correlations in the .5 to .6 range. Subjective live estimates of rib-eye area; percent kidney, pelvic and heart fat; and carcass grade gave lower correlations; i.e., in the .3 to .5 range. In this study it was indicated that market interests can subjectively estimate percent trimmed, boneless retail cuts from the round, loin, rib and chuck (cutability) with precision adequate to justify use of the dual grading concept when working with groups as large as five cattle. (AH d1-12)

To summarize, it appears that progress is being made in this area with the human eye, trained in what to look for, being almost as effective as the various mechanical and electronic devices studied to date.

In two studies on variability in yield of trimmed retail cuts, it was found that live and/or carcass weight accounted for more of the variation than other carcass variables. (AH d1-22, AH d1-12)

In one of these studies a correlation of .94 was obtained between live weight and pounds of retail product after adjusting for age and breeding group

effects. Carcass weights and measurements added little to age adjusted live weight in predicting pounds of retail product. The interpretation that can be put on these results is that variations in age adjusted live weight are appreciably greater than variations in carcass composition on the same basis. Thus, live weight differences at the same age seem to account for most of the variation in pounds of retail product on a within breeding group basis. (AH d1-12)

- 2. <u>Conformation scores</u>. In a study of classification (conformation evaluation) in cattle of reproductive age, it was found that classifiers differ appreciably in their repeatability of evaluations and that repeatability tends to be lower when evaluating younger cows. Some classifiers had a correlation of .9 or greater in successive scorings of the same animal at different times. (AH d1-31)
- 3. Growth rates. Additional studies have been made of effects of sex and age on performance to weaning. One of these on Virginia BCIA data involved over 28,000 records in many herds. The results confirm the general validity of results previously summarized on smaller bodies of data from experiment stations in the same general geographic area and with the same breeds (Hereford and Angus). Upward adjustments of 10, 5, 3, 2, and 3 percent were found necessary to adjust calf weaning weights of calves from 2, 3, 4, 5, and 12 years of age and older dams, respectively, to the average of weights of calves from cows 6 11 years of age. (AH d1-7)

A study on cow and calf weights at the Texas Station, involving a large amount of data on Hereford, Brahman, and Hereford-Brahman crosses, indicated that there was a rather uniform increase in average calf weight as dam weight increased up to 1050 pounds, a leveling-off of calf weights when dam weights increased from 1050 to 1300 pounds, and a decrease in calf weights as cow weights increased up to 1350 pounds. It appears that weaning weight selection would be more efficient if dam weight were taken into consideration.

In an analysis of a large volume of Oklahoma data on environmental effects on preweaning traits, it was found that the sex effect was greater in creepfed than in noncreep-fed calves. However, the age of dam effects were quite similar in both creep-fed and noncreep-fed calves. (AH d1-31)

From twin data, birth weight, butter fat production from 0 to 60 days, creep feed consumed and sex had a highly significant influence on weaning weight; the standard partial regression coefficients ranging from .30 to .42. (AH d1-43)

Postweaning gains made during the first 28 days on feed were more highly correlated with total gains than was any other gain period. The effects of initial age on postweaning gains were negligible while the effects of initial weight were more important in steers started on feed at 13 and 18 months of age than in steers started on feed at 8 months of age. (AH d1-10)

A limited amount of data continued to indicate the feasibility of producing 1000-pound calves in a year or less. The goal of producing 1000-pound calves at weaning is probably several generations in the future. However, data from the Texas Station show that a group of steers from various breed groups, which were managed and fed to gain a maximum, averaged 963 pounds at 365 days of age, as compared to 973 pounds last year. Some of the breed groups exceeded 1000 pounds at one year of age. (AH d1-22)

Performance test information is being gathered in virtually all research herds. In several instances, sires are progeny tested before they are used extensively in selection herds. At the Alabama Station, a total of twelve 140-day postweaning performance tests have been completed. During the first 10 years, a total of 517 bulls completed the test with an average daily gain of 2.24 pounds and a weight per day of age of 2.06 pounds; while during the last two years, 139 bulls completed the test with an average daily gain of 2.42 pounds and a weight per day of age of 2.29 pounds. The top third of the bulls gained an average of 2.73 pounds daily during the last four years, as compared to the lowest third which gained 2.14 pounds daily. These bulls brought an average sale price of \$775.00 and \$475.00, respectively. Weight per day of age was also evidently considered in buying bulls, since the top third, with a weight per day of age of 2.46 pounds, brought an average of \$767.00 per head, as compared with 2.09 pounds and \$482.00 per head for the lower third. (AH d1-29)

At the Tennessee Station, two methods of developing and testing herd bulls from weaning to approximately 20 months of age are being compared. During the past year, 41 Hereford and Angus bull calves from various Tennessee substations were used in this experiment. Representative bulls from each method will be progeny tested on random groups of cows to determine which method serves as a better indicator of the progeny's growing ability. (AH d1-9)

It appears from Arizona data, in which cattle were maintained year round on the range, that weight after stress might be about the best single growth trait for which bulls could be selected. This was based on the expected responses of other growth traits to such selection. Data on heifers indicate that fall yearling weight would be the best single growth trait on which to base selection. (AH d1-14)

C. Genetics and Interrelationships of Performance Traits

1. Heritability of quantitative traits. In a study with identical and fraternal twins, heritabilities were found of .80 for feed efficiency, .88 for 8-month milk production, .90 for 8-month fat production, .45 to .91 for several body measurements, and 0 for age at first calving. With the exception of age at first calving, these estimates are considerably higher than those usually found in non-twin herds. (AH d1-43)

In an extensive study utilizing data from breeders' herds in Virginia, heritabilities of preweaning average daily gain, weaning grade and an index

based on both gain and grade were found to be near 35 percent for Angus calves - again close to experiment station estimates. Heritabilities for Herefords were slightly under 30 percent. (AH d1-7)

A heritability estimate of .11 for pounds of carcass fat adjusted for differences in carcass weight was found. The estimate for pounds of carcass lean on the same basis was .0. Thus, these results suggest that there is not a great amount of genetic variation in composition on a weight constant basis when evaluating cattle of the same general type of the same breed. In the same study, estimates of heritability for growth traits, conformation score, marbling and rib-eye area were all high; i.e., in the .4 to .6 range. The genetic correlation between final weight and marbling score was positive and the genetic correlation between final weight and weight adjusted pounds of carcass fat was a high negative.

In an analysis of data from the same source an attempt was made to predict pounds of edible portion of the carcass from certain blood constituents. In a multiple correlation study an R^2 of approximately .5 was obtained between several blood constituents and pounds of edible portion. (AH d1-13)

- 2. <u>Genetic correlations</u>. In an analysis of growth and carcass data no major genetic antagonisms were indicated between the traits that contribute to productive efficiency and desirable carcass traits. However, in this study a high positive genetic correlation was obtained between outside fat and carcass grade (marbling). The phenotypic correlation between outside fat and carcass grade was a low positive while the environmental correlation was negative. (AH d1-31)
- 3. Genetic-environmental interactions. Data were studied from 104 steers slaughtered in 1962 and 1963 in a North Carolina study in which bulls are bred artificially to cows in each of three herds in different geographic areas of the State.

Twenty-five items relative to live weight carcass measurements and cooked steaks were considered in the analyses. Main effects; i.e., location, ration, sire, and year, were significant for most carcass measurements, and ration significantly affected taste panel scores for juiciness and flavor. Interaction between ration and year was significant for percent dress, marbling score, and carcass grade. The only indication of a genetic-environmental interaction was in percent separable lean in the 9-10-11th rib cut. Heifers which are progeny of sires used in this project are calving for the first time in 1964, and data on cow performance will soon be available for further consideration of interactions. (AH d1-23)

The genetic-environmental interaction study at Brooksville, Florida, which is an interregional cooperative study between that station and Miles City, Montana, is now in its third year. Adaptation of cattle from one station to the other has been relatively good, although some differences in shedding of hair and growth rate of calves has been noticed. (AH d1-41)

4. Genetic defects. A study of snorter dwarfism, as well as other types of dwarf anomalies in beef cattle, is being continued at the Florida Experiment Station. Dwarf x dwarf, dwarf x carrier, dwarf x normal, and normal x normal matings have been made in order to study the biochemical abnormalities in body fluids and tissues which may serve to identify carriers of the dwarfism trait. Embryos have been removed at 40, 60, and 90 days of age and the cows returned to the breeding herd to be bred again. This is an attempt to bracket the stage of development when the dwarf gene action is apparent. At present, 18 embryos of varying ages have been recovered and are being studied. Techniques are still being perfected for the culture of bovine leucocytes for cytogenic studies. (AH d1-34)

Dwarfism research is being continued at the Tennessee Station in a limited way. Known dwarf tester cows have been assembled and are being used to check herd sire prospects for possible dwarf genes. (AH d1-9)

In an analysis of data involving the profilometer technique for identification of carriers of snorter dwarfism a product moment correlation of 0.36 was obtained between predicted and actual genotype. This was significant at the .05 level of probability. However, this level of accuracy is considered too low to be useful in identifying genotypes relative to the snorter dwarf gene. (AH d1-13)

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BEEF CATTLE - PHYSIOLOGY Animal Husbandry Research Division, ARS

Problem. The loss resulting because of poor reproductive performance is hard to estimate. Data available from a 200 cow herd over a four-year period indicates that 10,490 potential pounds of calf were lost for each 100 cows bred. Thirty percent of the loss was because cows calved late and 70% because cows failed to wean a calf. Failure to show heat in the early part of the breeding season, or cows not settling on first service were the reasons for late calving. Most cows not weaning a calf in the fall were never diagnosed pregnant. These cows either did not show heat or they were bred and did not settle. Methods must be developed so that a higher proportion of cows will calve and will calve over a shorter period of time. In order to accomplish this, all cows must show estrus the first 21 days of the breeding season and a high proportion must settle at first service. This cannot be accomplished unless more information on physiological processes involved is available.

USDA AND COOPERATIVE PROGRAM

The program at the present time is mainly concerned with methods of improving, controlling or altering reproductive performance by hormonal, nutritional or other methods. It is carried on by physiologists and animal husbandmen at Beltsville, Md., and at the Department's Fort Robinson, Nebr.; Miles City, Mont.; Jeanerette, La.; and Fort Reno, Okla., stations in cooperation with the respective State experiment stations. Studies on the causes of reproductive failures are conducted with the herds at all these locations. gations on the relationship between reproductive performance and protein and energy intake levels are in progress at Beltsville, Fort Robinson, Jeanerette, Miles City, and Fort Reno to determine the relationship between anatomy of the pelvis and calving difficulties. Studies are underway at Fort Reno and Fort Robinson on control of the estrous cycle. Work at Fort Reno is designed to determine methods for altering the onset of post-partum estrus. Work at Miles City is designed to determine methods for inducing twin ovulations and thus twin births in beef cattle. Other studies at Fort Robinson include causes of maintenance and regression of the corpus luteum and methods to hasten the onset of puberty.

The Federal scientific effort devoted to research in this area totals 2.6 professional man years of which 2.2 are devoted to physiology of reproduction and .4 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

In addition to the research reviewed under Physiology in the Animal Biology problem area, several stations are conducting investigations designed specifically for beef cattle. These studies include the effect of controlled temperature and hormones on reproduction in heifers of both Brahman and

British breeding, the nature of sterility in animals which leave herds because of failure to reproduce, and research designed to more clearly define fundamental principles related to ova transfer and to develop a technique for collection and transfer of ova without surgery. Investigation of physiological effects of various hormone substances and development of simplified methods for bringing groups of animals into estrus within a short period is the objective of other research. Basic studies will determine the site of maturation of sperm and if proven to take place in the uterus, attempts will be made to isolate the material responsible for maturation.

Research in environmental physiology includes developing biological measures of response to environmental stress under controlled conditions, procedures for measuring environmental responses under field conditions, and the effect of nutrient restriction following weaning on the growth of heifers and upon subsequent lifetime production.

Other studies seek to explain the action of hormone compounds in promoting growth and the effect of levels of milk and forage intake at different periods on gain and weaning weight of beef calves.

The total State scientific effort devoted to beef cattle physiology research is 4.8 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Physiology of Reproduction

1. Nutrition and reproduction. Studies are being conducted at all stations to determine the effects of different levels of energy on the reproductive performance of beef females. At two stations, heifers were fed different levels of feed in drylot before and after calving. Death loss of calves was high in the extremely fat heifers while little difference in loss was noted between heifers on low levels of feed and those on a moderate level. The proportion of heifers becoming pregnant was increased when heifers were on a high level of feed following calving. Onset of heat following calving was delayed in heifers receiving the low levels of feed prior to calving. Although the proportion becoming pregnant in a 60-day breeding season was as high or higher for heifers fed the low levels as it was in heifers fed the high levels of feed prior to calving. The interval from calving to first heat was decreased when an increased level of energy was fed for 45 days before calving. (AH d1-37, AH d2-22)

Studies of various levels of supplemental feeding on range are being conducted at three stations to determine the effects on reproduction in beef heifers. Birth weight of calves from heifers on the low levels of feed were lower compared to calves from heifers on a higher level of feeding. The breeding date for heifers was delayed by low nutrition. (AH d2-12, AH d1-33, AH d2-34)

An experiment has been conducted at Front Royal Beef Cattle Research Station to assess the value of feeding or injecting vitamin A on calf survival. Neither the dam's supplementation nor the injection of calves with vitamin A influenced the post partum survival of the calf. (AH dl-4)

2. <u>Inducing early puberty</u>. At Fort Robinson, 21 prepuberal heifers were treated with synthetic hormones to determine if puberty could be induced early. All heifers except one receiving estradiol valerate either intramuscularly or intravenously, showed heat. However, only one heifer ovulated and regular estrus cycles were not established in any of the heifers. One heifer out of five receiving the alpha estradiol and the hydroxyprogesterone cap roate showed heat. Ovulation was not induced in either case and a regular cycle was not established. (AH d1-37)

At Jeanerette, 16 heifers were injected with progesterone and estradiol. Thirteen of these heifers showed heat at the end of the treatment. (AH d1-30)

- 3. Losses at or near calving. Studies to determine the association between pelvic opening size, calf size and calving difficulty have been conducted at Fort Robinson and Miles City. Summarization of these data show that calving difficulty can be predicted quite readily in heifers having their first calf at 3 years of age; while prediction in heifers calving at 2 years of age is rather uncertain. Data from Hereford dams indicate a high correlation between calf birth weight and body measurements. Male calves were larger than female calves in all measurements taken. (AH d1-37, AH d1-33)
- 4. <u>Inducing multiple ovulations</u>. An exploratory study of the effects of insulin and gonadotropin (PMS) on ovarian activity in beef heifers has been conducted at Miles City. PMS treatment increased: number of follicles 75 mm. diameter (1.5 vs. 5.8, P<.01); ovarian follicular fluid weight (1.76 gm. vs. 7.04 gm., P<.01); and number of corpora lutea (1.00 vs. 3.67, P<.05) all figures no PMS vs. PMS, respectively. Insulin treatment main effects were non-significant as were all insulin x PMS interactions. However, there was an indication that concomitant insulin treatment with the PMS reduced the individual animal response variation in ovarian activity obtained when PMS was given alone. (AH d1-33)
- 5. Control of estrus. Synchronization of heat has been studied at Fort Robinson and Fort Reno by feeding oral progestins alone or in combination with stilbestrol, and by injecting progesterone and estradiol valerate. Feeding Provera (Upjohn and Co.) for 18 days successfully synchronized heat in a 4-day period for heifers on a high level of feed but not in heifers on a low level. Conception rate was low. Feeding Droxone (E. R. Squibb and Co.) for 20 days synchronized heat in a 3-day period. Conception rate at first service was low. Studies indicate that this is the result of a low fertilization rate. Length of heat period and time of ovulation were also affected by synchronization. Attempts to decrease the amount of Droxone needed by addition of stilbestrol to the Droxone were not successful. However, it was demonstrated that heat could be successfully synchronized by feeding Droxone

for 9 days if heifers were given a single injection of estradiol valerate. Preliminary results indicate a conception rate comparable to the controls.

Heat was also successfully synchronized by 12 daily injections of progesterone and estradiol valerate. (AH d2-12)

The influence of small injections of estradiol-17 beta on ovarian activity was studied in 120 cycling heifers. Injections ranged from 20 mcg. daily to 640 mcg. daily. All levels of estrogen reduced luteal activity and follicular growth and development. The inhibition of ovarian activity was approximately proportional to the level of estradiol administered. (AH d1-37)

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BEEF CATTLE - NUTRITION AND MANAGEMENT Animal Husbandry Research Division, ARS

Problem. Producers of beef cattle need improved feeding methods which will result in optimum pasture and feedlot gains, reduced feed consumption per pound of beef produced, optimum reproductive rates and desired carcass qualities. To meet these needs basic nutritional information is required such as: When should beef animals be fed for maximum gains and when for more limited gains? What nutrient combinations produce rapid growth of muscle with a minimum of fat deposition? How may breeding animals be economically raised that will be capable of a high level of reproductive performance over a long lifetime? What are the nutritive contributions made by range and pasture and what supplementation is required when they are used? Research is also needed on the relation between animal production and types of shelters and equipment, feeding systems, and methods of increasing labor efficiency.

USDA AND COOPERATIVE PROGRAM

This is a continuing program carried on by nutritionists, biochemists and animal husbandmen on basic and applied problems related to feeding and management of cattle for beef. The work is in progress at Beltsville, Maryland; in cooperation with State Experiment Stations at federally owned stations in Miles City, Mont.; Fort Robinson, Nebr.; Fort Reno, Okla.; Jeanerette, La.; Brooksville, Fla.; and Front Royal, Va.; and in cooperation with State Experiment Stations at Tifton, Ga.; College Station, Tex.; and Newell, S. Dak.

The <u>Federal</u> scientific effort devoted to research in this area totals 12.2 professional man years. Of this number, 4.2 are devoted to <u>digestion and metabolism</u>; 1.4 to <u>concentrate</u>; 1.7 to <u>forage preservation and utilization</u>; .5 to <u>nutrient requirements</u>; 2.1 to <u>range and pasture management</u>; .8 to <u>management practices</u>; and 1.5 to <u>program leadership</u>.

There is one grant involving Public Law 480 funds with the Agricultural College in Poznan, Poland. The project is to determine the trace mineral content of forages as affected by stage of growth and methods of harvesting and storing. The project is to run five years (1963-1968) and is supported by \$47,311.66 equivalent in Polish zlotys.

There are contracts totaling \$251,754 with the Agricultural Experiment Stations at California, Kentucky, Florida, Wisconsin, and Nebraska. These projects are concerned with the methods of feed preparation and level of grain in the ration, the sites and amount of starch utilization in the ruminant digestive tract, ration components which control feed intake, graded levels of energy intake upon reproductive performance in beef cattle and management of males of beef and dairy breeding for beef production. These projects will run three to four years (1964-1967 or 1968), their basic purpose being to determine the potential for increased utilization of grains for beef production.

PROGRAM OF STATE EXPERIMENT STATIONS

The States have research in progress on the basic functions of the rumen, particularly the animal-feed interrelations which are responsible for bloat, efficient feed digestion, and the synthesis of essential nutrients. (Additional investigations of rumen function appear in problem area #1).

The basic requirements of beef cattle for specific nutrients, their metabolism, interactions and availability in feeds are receiving attention at a number of stations. Some of the topics being investigated are: (1) The requirements, metabolism, and interactions of the many major and trace nutrients. (2) The effect of feed additives or implants upon growth and feed efficiency. (3) The relation of nutrients to metabolic disorders. (4) The toxicity of molybdenum and fluorine. (5) The value of irradiated feeds and assimilation of fallout products. (6) The use of roughage concentrate ratios and chemical regulators for feed intake control. (7) The effect of physical form of the ration upon nutritional value. (8) A re-evaluation of the vitamin A requirements and factors affecting them made necessary by apparent deficiencies on rations with ample carotene based on earlier standards.

The efficiency of feed use is being improved through investigations of concentrate and forage feeding. The problems and advantages of all-concentrate or high-concentrate feeding have high priority at this time. The comparative values of various kinds and combinations of feeds and the effect of different physical forms (chopped, pelleted, etc.) are being studied. There is considerable emphasis on development of methods for evaluation of forage crops. Four cooperative regional research committees (NC-64, NE-24, S-45 and W-34) have all or a significant part of their project devoted to this evaluation.

Management problems are being investigated. The main topics are combinations of pasture and drylot feeding; maximum use of pasture throughout the season, especially extending the period by using late fall and early spring pasture crops; supplementation needed when low quality roughages are fed; and creep feeding vs. non-creep feeding of calves.

The State stations have 49.6 professional man years devoted to Beef Cattle Nutrition and Management.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Digestion and Metabolism

1. <u>Digestion techniques</u>. <u>In-vitro</u> tests with rumen fluid indicated that glutamic acid did not alter the ammonia production from urea and that glutamic acid was slowly deaminated by rumen microorganisms. Metabolic studies with steers indicated that the addition of glutamic acid to the "urea-purified" ration did not improve nitrogen retention over the basal ration.

Glutamic acid addition resulted in a significant increase in the molar percentage of butyric and higher volatile fatty acids in the rumen liquor. Glutamic acid appeared to be inferior to acetic acid, on an equal carboxyl basis, in its ability to lower excessive ammonia production in the rumen. (AH d2-8, AH d2-14)

When steers were fed loose or pelleted natural and purified rations, salivary flow and buffering capacity of the saliva was significantly depressed due to feeding the purified rations. Pelleting both the natural and purified rations resulted in a significant depression in buffering capacity of the saliva. The weight of the rumen contents was significantly depressed due to pelleting the rations, indicating a faster rate of passage on the pelleted rations. Pelleting also resulted in a significant depression in crude fiber digestibility with both rations. Ruminal pH, molar percentage of acetic acid, and ruminal ammonia concentrations were significantly less on the purified rations while total concentration of the volatile fatty acids was greatest on the purified rations. (AH d2-8, AH d2-14)

 $\mathrm{Cr}_2\mathrm{O}_3$ was used as a marker to estimate rate of passage of food residues through the digestive tract of (6 and 8 months) pregnant heifers assigned to four levels of feed intake (60, 80, 100, 150% of recommended levels). There appeared to be a faster rate of passage when the heifers were fed at the high levels of feed intake and an equal or slightly slower rate of passage during the later stage of gestation. (AH d2-11, AH d2-22)

- 2. Bloat in beef cattle. In a study designed to test the effects of the physical form of a bloat producing ration on performance and severity of bloat, average daily gains were increased and severity of bloat was decreased if steers were fed the ration with the alfalfa coarsely (1 1/2" screen) rather than finely ground (3/8" screen). Rolling rather than grinding the barley portion of the ration did not affect gains or the severity of bloat. (AH d2-13)
- 3. <u>Urinary calculi</u>. Cattle studies at College Station, Texas, in cooperation with Texas Agricultural Experiment Station indicated that the addition of ammonium chloride prevented the formation of urinary calculi when feeding a basal ration of high moisture sorghum heads regardless of the other supplements used. Dipotassium phosphate, injected vitamin A plus dipotassium phosphate or diethylstilbestrol, increased the incidence or quantity of calculi while synovex had little or no effect. A dicalcium supplemental phosphate reduced incidence and quantity and improved performance. (AH d2-31)

In an attempt to produce urinary calculi of the magnesium-phosphate type, varying levels of calcium, phosphorus, potassium, and sodium were fed to albino rats. A high incidence of calculi occurred when a diet containing .3% Ca, 1.20% P, .1% K, and .8% Na. Urolithiasis did not occur when the percentage of any mineral varied markedly from those given above. Some kidney damage was observed when the diet was low in calcium and high in phosphorus. (AH d2-31)

4. Pesticide residues. At Tifton, Ga., steers fed for 27 days on corn silage made from corn with no dimethoate, with 1.1 parts per million and with 6.7 parts per million dimethoate (field applications were 1 and 2 lb. per acre, respectively) showed no effects from consuming 50 lb. of the treated silage per steer per day. The dimethoate treated silage was as palatable as the untreated. Blood samples taken the day before the test and at 6-day intervals during the feeding period showed no difference in the blood choline-sterase activity. This test and the concurrent one with dairy cattle showed dimethoate to be a pesticide that was not harmful to beef and dairy animals when used on forage to be ensiled for feeding. (AH d2-32)

<u>In-vitro</u> tests subsequent to those reported previously have confirmed the opinion that rumen protozoa are useful as bio-assay tools for screening insecticides which are susceptible to microbial breakdown and may be used on forage and other cattle feed crops. (AH d2-32)

- 5. Microbiology of the rumen. Concentrated cellular preparations of $\underline{\mathbf{I}}$. Prostoma were incubated for 4.5 hr. at 38° C. with 1.0 µc each of linoleic-l-C¹⁴, containing 250 µg/ml. of dihydrostreptomycin sulfate. After incubation, linoleic-l-C¹⁴ activity was distributed as follows: sterol and sterol esters, 0.4%; triglycerides, 13.3%; diglycerides, 6.4%; monoglycerides, 8.8%; cephalins, 12.7%; lecithins, 56.6% and other lipids, 1.8%. Oleic-l-C¹⁴ activity was primarily concentrated in triglyceride (24.0%) and the lecithin phospholipids (47.7%). Stearic-l-C¹⁴ activity was concentrated primarily in the triglyceride component (61.1%). This information may be helpful in further understanding the protozoan's metabolic requirements for future axenic culture studies, and its role in lipid contribution to the ruminant host. (AH d2-26)
- 6. Anatomical and physiological factors affecting digestibility. Total daily salivary production ranged from 38 to 68 liters per day when steers were fed a ground and mixed Bermudagrass corn ration and ranged from 25 to 51 liters per day when alfalfa pellets were offered. Animal and ration differences were significant. The steers secreted significantly more saliva (3X) while eating than while resting. When not feeding, the rate of salivary secretion was relatively uniform throughout the day. (AH d2-8)

As determined by feeding calves \underline{per} os and via abomasal fistulae, post ruminal fiber utilization was about $\overline{25\%}$ less than when exposed to digestive action in the rumen. (AH d2-8)

B. <u>Concentrates</u>

1. High concentrate rations for finishing steers. Yearling steers fed an all concentrate ration based upon ground shelled corn and urea gained more rapidly when implanted with 24 mg. of stilbestrol. Gains were not improved when the ration was fed as a pellet or with a magnesium zinc supplement. However, feeding the pelleted ration resulted in steers with more fat over the rib eye, darker ruminal epithelium and higher ruminal ammonia concentrations. (AH d2-14)

At Fort Reno, Okla., fine grinding of milo resulted in a 5.2% improvement in feed efficiency compared to coarse grinding. Rate of gain was nearly the same. The use of finely ground milo (1/8" screen) in fattening beef cattle rations should be considered, especially in high roughage rations. It may be less desirable in high concentrate rations. For example, if molasses or fat is not used, a coarsely ground or rolled milo may be most desirable due to the dustiness of the finely ground milo. (AH d2-28)

All-barley rations supplemented with soybean meal, calcium, and vitamin A were fortified with trace minerals. There was an improvement in feed intake, body weight gains, feed efficiency, carcass grades - especially with supplementation of cobalt, zinc and iron. Dehydrated alfalfa meal and molasses appeared to offer no advantage other than as a source of trace minerals. The need to fortify all-concentrate diets with trace minerals is indicated. (AH d2-28)

Three rations containing 65, 80, and 95% concentrates were evaluated by finishing steers to market weights in studies at South Dakota. Feed costs per pound of gain were similar among the different treatment groups. The most efficient gains and the best carcass grades were obtained on a ration containing 80% concentrate and 20% roughage. Steers from the lower levels of concentrate had meatier carcasses yielding a larger percent of boneless trim retail cuts.

Short yearling steers were finished during the summer on a ration consisting of ground mixed alfalfa prarie hay, 20% and rolled barley, 80%. One group was full-fed and the other fed 85% of the amount consumed by the full-fed steers. Full-fed steers required less feed per pound of gain and made slightly cheaper gains, and averaged slightly higher in carcass grades. (AH d2-35)

Weanling steers supplemented with 5 lb. of rolled barley per day gained 1.54 lb. per head daily while those fed no grain averaged .98 lb. per head daily. The type of roughage (hay or silage) fed had no influence on the average daily gains. There was no significant differences in cost per lb. of gain.

In both yearling and steer calf groups, lots receiving long hay in addition to a ground and mixed ration consumed more total feed and gained faster (16%) but did not make more efficient gains. There were no differences in slaughter or carcass grades.

Rations containing about 70, 63, and 52% concentrates with or without coastal Bermudagrass hay were fed to steers at Tifton, Ga. Feed consumption increased as level of concentrates decreased. Gains were greatest for steers consuming the medium level of concentrates but overall steer performance was similar for the medium and high concentrate levels. There was no advantage to offering unground hay. Steers offered such rations can be placed on full feed the first day.

Low groups of 10 steers each were fed grain to roughage ratios of 9:1, 8:2, 7:3, and 6:4, respectively. The grain mixture was cracked shelled corn and fortified protein supplement. The roughage was coastal Bermudagrass hay. Daily gains decreased as the proportion of concentrate in the ration decreased. There was little difference in carcass grades or dressing percent between groups. Steers fed the highest concentrate to roughage ration were considered to be the most profitable. (AH d2-14)

C. Forage Preservation and Utilization

1. Utilization of coastal Bermudagrass. Six systems of utilizing coastal Bermudagrass were evaluated in steer feeding and pasture trials at Tifton, Ga. The average daily gains for the various systems were: 1.25 lb., continuous grazing; .93 lb., rotational grazing; .98 strip grazing; 1.94 lb., feeding pellets; 1.61 lb., feeding dehydrated hay; and 1.01 feeding fresh chopped grass. In general, feed consumption figures (where available) are correlated with gains. (AH d2-3)

From steer feeding tests at Tifton, Ga., it was concluded that dehydrated and suncured Bermudagrass pellets were equivalent in feeding value when offered in a 30 or 60% Bermudagrass ration. Steers receiving the 30% Bermudagrass ration (more corn) made faster and more efficient gains. (AH d2-28)

During a 75-day feeding period, steer calves gained about twice as fast on "poor" quality Pensacola Bahiagrass hay as when fed coastal Bermudagrass hay (.89 vs. .41 lb.). Steers fed good quality Bahiagrass gained 1.05 lb. per day. Chemical analyses revealed little differences among the hays. (AH d2-3)

2. Feeding value of pelleted feeds. At Beltsville, behavior studies have shown that more time was spent at the feeder when the steers were penned individually as compared to when penned by pairs. Feed consumption increased slightly and there was a significant increase in the speed with which the feed was consumed when the steers were penned by pairs. Time of day, form of feed and body weight relationship to feeding behavior patterns were the same as reported previously. It also appears that environmental temperature may be related to feeding behavior. Preliminary studies suggest that cattle feeding patterns are directly related to light dark cycles. AH d2-28)

Acid detergent fiber (ADF) of a legume (alfalfa) and a grass (timothy) is being studied in a metabolism trial designed to determine if there is a forage species difference or an effect of the form in which the forage is fed (pelleted or ground) that may be related to the use of ADF as an indicator of digestibility. (AH d2-28, AH d2-14)

D. Range and Pasture Management

1. Range supplementation studies. At Brooksville, Fla., cows fed a year-round daily supplement of 5 lb. of blackstrap molasses with added urea (15% protein equivalent) were compared with cows which received the molasses-

urea mixture free choice during the winter. Calf performance to weaning showed no essential difference between the groups; however, the slight difference was in favor of the winter feeding of the cows. (AH d3-2)

Range management studies (Miles City, Mont.) have indicated that both cow and calf gains during the spring period were higher on the introduced grass pastures of crested wheatgrass and Russian wild-rye. Calf gains were similar on the introduced grasses. It will not be known until fall (1964) if the advantage in spring gains will carry over until weaning. The calving percentage of 94.6% was considered very satisfactory since no winter supplemental feed was provided and only a small amount of 2-year-old grass hay was fed during calving. It was concluded that optimum calving performance from range cows can be obtained if the range is kept in good condition. (AH d3-1)

At Tifton, Ga., average birth weights and weaning weights of calves were greater (62.4 vs. 81.4 lb. and 448 vs. 500 lb.) when the cows were pastured on burned range only in the spring and improved pasture only in the summer as compared to when pastured on unburned range and 0.6 acre improved pasture per cow in the spring and summer. (AH d3-3)

Studies on pasture and timber integration resulted in average liveweight gains per acre of pasture of 220, 219, 194, and 147 lb. for Bahiagrass, coastal Bermudagrass, Dallisgrass and Carpet grass pastures, respectively. Based on tree spacings, the liveweight gains were 248 lb. for no trees, 190 lb. for 20 x 20 tree spacings and 177 lb. for 12 x 12 spacings. (AH d3-4)

A cooperative experiment is now in progress where sheep, cattle and cattle plus sheep (1:5 or 1:1) are grazed at two stocking rates. The preliminary data suggests that there may be higher gains and more meat produced per acre when the two species are grazed together in a 1:5 ratio. (AH b3-10)(also see area 15-C-1)

E. Management Practices

1. Management of cattle and pastures for beef production. Graded levels of thyroprotein (0 to 15 1/2 g./day) were fed to intact and ovariectomized heifers at Fort Robinson, Nebr. Gains decreased as thyroprotein intakes increased and the intact heifers gained more than the ovariectomized heifers at all treatment levels. (AH d2-21)

At Fort Reno, Okla., grass hay, sorghum silage, and corn silage were fed to weanling heifers (for a period of 105 days). The weanling heifers gained uniformly during the feeding test and the condition and grades at the end of the trial were similar. More sorghum silage than corn silage was required for weanling heifers to make similar gains. Condition scores of the heifers were practically identical and there were no significant differences among the three treatments. (AH d2-34)

Hereford heifers were used to reflect the change in major tissue growth and development from 9 to 18 months of age. Bone made only a small change, while lean increased from 71 to 124 lb. Fat increased rapidly throughout the fattening period.

Expressed in terms of percent, lean and bone in the carcass decreased while fat increased. The percent lean in a carcass from animals slaughtered at 18 months of age was about 10% less than at 9 months. Fat increased by 12.3% and bone decreased by 2.4%. (AH d3-7)

The trace element study of soils and forages in Poland shows that there is a wide range in the mineral composition of soils of the experimental plots and significant differences between samples taken at different soil levels. (E21-AH-6)

Two trials (Fort Reno, Okla.) were conducted to study the effects of varying the level of winter feed and varying this level from low to high and vice versa during the first winter as weaner calves or the second winter as bred yearlings on subsequent performance of replacement beef females. In one trial, all the heifers were continued on the moderate level for the second calf crop.

The results show that the main effect of the first winter at a low level is to delay the onset of puberty and, as a result, delay conception and average calving data as two-year-olds. Other than this, the effects on growth and later performance appear negligible, providing good pasture and adequate feed levels are provided thereafter. There appears to be no permanent effect of a low level during one winter as a calf.

Of the two winter periods, low level feeding the second winter as a bred yearling appears to be most damaging. Here the heifer, while still continuing to grow and develop, must undergo the strain of calving and early lactation on inadequate feed supplies. The results are to delay re-breeding for the second calf, and a sharp reduction in milk flow. Naturally, this reduces the weaning weight of the calf.

Neither the low-high nor high-low sequence appears to be as beneficial as an adequate feed level (moderate) each winter in terms of growth and reproductive performance. (AH d2-36)

Eighty bred yearling Hereford heifers were fed four different levels of winter supplemental feed (milo and cottonseed meal) while grazing dry native grass pastures. Two groups were fed at a low level prior to calving and two groups were fed at a high level prior to calving. The heifers of one group on each level were switched to the opposite feed level at calving time. Thus, the four treatments were: low-low, low-high, high-low, and high-high, indicating, respectively, the feed levels before and after calving. The low level was the amount of feed necessary to establish a loss of more than 20% of the fall weight through calving. The high level was the amount necessary to maintain the fall weight through calving.

Heifers fed at the high level prior to calving dropped calves that averaged 14 lb. heavier at birth than did the low level heifers. Heifers fed high prior to calving returned to heat earlier, bred back earlier and had a high conception rate (93% and 100% vs. 53% and 75%) than did heifers fed low up to calving. Raising the low level heifers to high at calving resulted in a 2-week earlier breeding date, a high conception rate (75% vs. 53%) and an 18 lb. heavier average weaning weight. Average weaning weights followed amount of winter feed fed and were as follows: high-high, 432 lb.; high-low, 408 lb.; low-high, 376 lb.; and low-low, 358 lb. The heifers of the high-high lot had the highest average milk production, the heifers of the low-low lot had the lowest and the other two lots were intermediate between the two extremes. (AH d2-12)

2. Beef production from beef, dual purpose, and dairy steers. In a cooperative experiment, Holstein steers outgained Jersey and Hereford steers at all feeding levels studied. When placed on a higher feeding level, all steers that were started on a lower level of feeding gained more rapidly and more efficiently than steers that were liberally fed initially. Tenderness and palatability of the meat, and fat and lean content were related to the feeding regimens just prior to slaughter. (AH d3-6) (also see Area 5-C-5)

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PRODUCTION INFLUENCES ON BEEF Animal Husbandry Research Division, ARS

Problem. Beef, lamb, pork, and poultry are excellent sources of wholesome and digestible animal proteins and fatty acids necessary in maintaining a healthy, appetizing diet. However, these meats must be of high quality, as well as in plentiful supply, if they are to retain their high position and esteem in the minds of consumers. Proper finish, a high proportion of lean, with adequate intramuscular fat, tenderness, full flavor, and color desired by the consumer are the goals the meat producer must strive to attain through breeding, feeding, and management. The quality of cuts and kind of meat are directly reflected in the demand and in the price of the product.

Egg shell strength and yolk quality, strength of wool, fatness, quantity, flavor, color, and tenderness of meat are all known to be influenced by production practices. However, these quality characteristics and many more are not well understood, even though they are of considerable economic importance. Effective measures of evaluating quality differences are of great importance in determining the nature and effect of production practices on the products.

USDA AND COOPERATIVE PROGRAM

This is a continuing program conducted by food product technologists, wool and fiber technologists, biochemists, chemists, physiologists, statisticians, and animal husbandmen engaged in both basic and applied research designed to develop methods and information which will be useful in evaluating quality and quantity of animal products and will be useful in aiding and directing livestock production. Research on beef, veal, lamb, and pork is directed at the influence of selection and breeding, nutrition, physiology, management, and other production variables on carcass and meat quality and quantity. Standards are being applied and adapted for appraisal of slaughter animals, of carcasses, and of meat cuts. The objective of the work with poultry and eggs is to ascertain those factors of nutrition, breeding, and management which contribute to the initial quality of poultry products and their capacity to retain that quality. Studies with wool, fur, and fiber are conducted to determine the physical, chemical, and biological structures and properties of wool and other animal fibers as influenced by production factors. Research on humane slaughter was continued on a reduced scale, primarily to bring to a conclusion some phases of electrical immobilization and physiological responses. The work is conducted at Beltsville, Maryland; Dubois, Idaho; Fort Wingate, New Mexico; and in cooperation with eight State experiment stations. Cooperation is also carried out with the Eastern and Western Utilization Research and Development Divisions, the Human Nutrition Research Division, the Agricultural Engineering Research Division, and the Market Quality Research Division.

The Federal scientific effort devoted to research in this area totals 15.6 professional man-years. Of this number 5.5 are devoted to beef; 1.1 to lamb, mutton, and chevon; 4.0 to pork; 1.0 to poultry and eggs; 2.1 to wool, fur, and fiber; 0.5 to humane slaughter; and 1.4 to program leadership.

A grant with the Polish Academy of Sciences in Poland provides for studies on the color of pork as influenced by heredity, sex, age, feeding, and management. Its duration is for five years (1960-1964) and involves PL 480 funds with \$42,784 equivalent in Polish zlotys.

PROGRAM OF STATE EXPERIMENT STATIONS

Beef. The influence of feeding and management treatments on carcass and meat characteristics include fattening on grass, drylot, or combinations of these; varying the length of heavy silage feeding preceding finishing with a high-energy ration; creep feeding versus no creep feeding during the nursing period, and various combinations of ration ingredients with and without adjuvants. Many of the projects include economic considerations as well as consumer acceptance and laboratory analysis for quality. Regional project NC-58 is designed to objectively identify the factors that characterize differences in beef carcasses, evaluate the relative importance of these factors and find the best indicators of these carcass traits in the live animal.

A number of breeding projects contributing to regional research projects NC-1, W-1, and S-10 are designed to determine the effectiveness of selection in improving carcass traits. Other independent studies include the importance of beef conformation as contrasted with dairy type in the production of consumer acceptable beef.

The total State scientific effort devoted to production influences on animal products research is 51.4 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Beef

1. Tenderness. Biopsy samples were surgically removed from the right side of 35 six-month-old calves, 32 nine-month-old calves, and 39 twelve-month-old steers, and refrigerated 48 hours. Muscle tissue was heated to doneness in deep fat and tested with the slice tenderness evaluator (STE) for tenderness. A low relationship was noted between the STE shear values of the biopsy samples and those of the 9-11 rib roasts from the carcasses of the mature animals. Ninety-one percent of the biopsy samples had STE shear values higher than those of the matching roasts or 19.0 and 11.8 pounds, respectively. Part of this difference may have been influenced by the animal's later nutritional regimes and greater degree of finish. (AH d4-4)

Continued study of refinements in methodology and usefulness of the STE as an objective tenderness testing method indicated that the meat slice need not be held in position by the holder place. Operating time was reduced one-third by eliminating use of the holder. Under present operating procedures, the STE measurement efficiency and accuracy compare favorably with the Warner-Bratzler shear. Correlations between STE shear values and panel tenderness score usually range from -.74 to -.85 for beef samples. (AH d4-4)

In a study of beef roast slices from the heated 9-11 rib roast from 136 beef carcasses, it was found that the three dorsal locations of the muscle cross-section were the most tender; while the medial and lateral locations were 5% tougher. The variance between locations of the muscle cross-section strongly emphasizes the need for careful selection and control of sampling when testing for tenderness. (AH d4-4)

Steak and 9-11 rib roast samples from 59 steer and cow carcasses, grading Choice + to Utility - and maturity groups ranging from A+ through F (15 to 80 months of age) have been tested by a palatability panel. Certain animals appeared to have more palatable roasts than steaks, but in others the reverse was true. These irregularities occurred more frequently among carcasses of the older maturity groups. The samples from the younger beef carcasses (maturity group A+ through B+) showed no difference in average palatability scores between roasts and steaks. (MQ 3-34)

In a study to determine specific palatability characteristics of beef samples, 48 loin and 9-11 rib samples were tested from 48 beef carcasses representing Shorthorn, Angus, and Hereford steers produced at the Beef Cattle Research Station, Front Royal, Virginia. The results indicated that loin samples (one-inch steaks) from Herefords had a more pronounced and desirable lean meat flavor than samples from the Shorthorn steer carcasses. Samples from the Angus and Hereford steer carcasses had a finer texture, were more tender, had a richer and greater quantity of juice and a higher overall desirability among their steaks than loin samples representing the Shorthorn breed. As in previous reports, the Shorthorn steers had the less tender meat. (AH d4-4 and MQ 3-34)

An analysis of the data obtained from the study of paper chromatographic separation of the tissue free-amino acids and their relationship to tenderness of beef muscle was made. The statistical conclusions were that a relationship does exist between meat tenderness and the amino acids represented by leucine-isoleucine. However, the relationship is not sufficiently high to warrant its exclusive use as an objective method for evaluating tenderness. It was useful in differentiating between rations. (AH d4-6)

A comprehensive statistical analysis of the data on 112 beef samples on free amino acids (leucine-isoleucine), hydroxyproline, the Warner-Bratzler shear, and the palatability committee shows that each measures differences in tenderness. The Warner-Bratzler shear accounted for 62% of the variation in panel tenderness and was the only objective measure significantly related to panel tenderness in rib samples from six-month-old animals. However, on those samples tested from beef after reaching slaughter weight, all three measures were significantly related to tenderness. The Warner-Bratzler, hydroxyproline, and leucine-isoleucine methods accounted for 22, 33, and 5% of the variance in panel tenderness, respectively. (AH d4-6)

Analysis of composite samples from 10 animals in which steaks from two muscles, longissimus dorsi and biceps femoris, were cooked to final temperatures (61, 68, 74, and 80° C), indicate a step-wise decrease of collagen nitrogen at each increased temperature. The percentages of decrease are similar for the two muscles. An initial study on the effect of steak size on cooking time to 80° C on shear value and on the bound water was completed. Small steak samples were cut to $1\frac{1}{2} \times 2 \times 1$ inch in size and weighed approximately 90 grams. Cooking times were reduced 17 and 20% and shear values were reduced 11 and 13% for the L. dorsi and B. femoris, respectively. It would appear that shear values are dependent upon cooking time as well as final temperature. The correlations between shear values for the larger (normal cut) vs. the smaller steaks was 0.87 for L. dorsi and 0.68 for the B. femoris. The correlations between cooking times for the two sizes of steaks was low. This would seem to indicate that animal variation was not a factor that influenced cooking time. (AH d4-5(c)

2. <u>Composition</u>. A majority of the ultrasonic research information on live cattle fatness and muscling has been obtained from measurements over the back and rib of the animal. Recent findings reported by this and other laboratories suggest that rib-eye area is not a good measure of total muscling of the animal. For this reason, efforts have been directed toward determining measurement areas capable of yielding more meaningful ultrasonic values of fatness and particularly muscling. (AH d4-7 and MQ 3-34)

The study undertaken last year to more effectively and accurately estimate live animal composition from linear measurements of the chilled carcass was expanded and continued. The volume of the total carcass, hindquarter (round + rump + loin), and the dorsal wholesale cuts (hindquarter + prime rib + chuck) were computed from five measurements, utilizing the basic definition of a triangular pyramid. Correlations between the calculated volumes for the three partitioned areas and the corresponding weights of the cuts were 0.78 or above. Carcass and round volume accounted for 90% of the variance in muscle and fat as determined by principal component analysis. This study is being continued and extended to include the live animal. (AH d4-7)

3. Quality and quantity of meat as affected by production

(a) Effect of sex. The data from yearling Hereford bulls and steers indicate that roast meat samples from bulls were substantially less tender, less juicy, lacking in flavor of lean, and from the color of lean less done than similar samples from steer beef when heated to the same degree of doneness. Part of these differences could be attributed to the differences in finish. Ether extract fat in the eye muscle of the 9-10-11 rib indicated that considerable variation existed in intramuscular fat when certain lines were crossed. The cross-line bull rib samples had more ether extract fat than representatives of either parent line. The data are being further examined for heterosis effects. (AH d4-7)

A preliminary analysis of data from an experiment comparing eating quality characteristics of bull and steer carcasses from identical twins indicated that steer carcasses contained more ether extract fat in the eye muscle, had a higher marbling grade, were more tender, and the overall desirability of the meat was much better. (AH d4-7)

(b) Beef from beef, dual-purpose, and dairy type steers. Holstein steers had the largest rib eyes (11.4 sq. in.) and the Jersey the smallest (8.9 sq. in.). However, when expressed in inches per hundred pounds carcass weight, the breeds representing the three types ranked as follows: Angus, Holstein, Jersey, and dual-purpose Shorthorn with values of 1.95, 1.82, 1.75, and 1.61 square inches, respectively. Feeding only hay to a portion of the steers resulted in less marbling of the loin eye and in a smaller total rib-eye area. However, expressed as area/cwt. carcass, the values were 1.88 inches compared with 1.80 for the other two rations. A ratio of lean meat to bone showed the Hereford steers to have the largest ratio, 3.69:1; followed by dual-purpose Shorthorns and Angus 3.60:1; Holsteins 2.97:1; and Jerseys 2.84:1. Beef-type steers had more extensive marbling deposits in the lean than did animals of the other types and higher slaughter and carcass grades. The palatability panel noted no breed differences in the 9-11 rib roast sample in desirability of aroma, but the flavor of lean for the Angus was one-half panel-unit higher than for the Jersey. No significant palatability differences were noted among 9-11 rib roast samples for the non-beef type steers for tenderness, juiciness, and overall desirability. Beef type steers rated slightly higher in these three categories than non-beef type steers. There were continued significant differences in eating quality of 9-11 rib roast samples from calves fed milk and calves fed milk replacer. Samples from calves fed milk were significantly more tender at six months of age than those fed replacer. However, when similar calves were fed to slaughter weight on concentrates or hay, they had recovered from any deleterious effects due to feeding the first six months on milk replacer. (AH d3-6)

(c) Electrical properties and quality of meat. A study was made to investigate the direct current electrical properties of fresh beef muscle at 3/4, 24, 48, 72, and 144 hours post-mortem. In addition to the usual electrical measurements of microamperage, millivoltage, and resistance, a technique was developed to measure the ability of the fresh tissue to retain an induced voltage. Data taken on 10 calves and 61 mature cattle indicated that tissue microamperage and voltage decreased sharply between 45 minutes and 48 hours post-mortem; while resistance and timevoltage measurements increased rapidly during the first 24 hours. When electrical properties were compared with palatability scores of the meat given by the panel, it was found that time-voltage values were significantly related to the overall desirability scores and tenderness values among calves and also to the juiciness scores among mature beef. Electrical resistance values were significantly related to one or more tenderness measures in calves and mature beef. Lower time-voltage curves and resistance values were associated with more tender and juicy meat.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Beef

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- Hiner, R. L. 1964. Slaughtering, cutting, and processing beef on the farm. Farmers' Bull. 1415 (Rev.) (AH d4)
- Hostetler, R. L. and Ritchey, S. J. 1964. Effect of coring method on shear values determined by Warner-Bratzler shear. (Manuscript submitted to J. Food Tech.) (AH d4-5c)
- Oltjen, R. R., Davis, R. E., and Hiner, R. L. 1964. Effect of urea, zinc, mineral buffers, stilbestrol, and pellets on the feedlot performance, carcass characteristics and blood proteins of cattle consuming all-concentrate rations based upon ground shelled corn. J. Animal Sci. (in press) (AH d4-7)

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- Thornton, J. W. and Hiner, R. L. 1963. Volume of beef round related to carcass composition. J. Animal Sci., 22(5):829. (Abstract) (Manuscript accepted by Journal) (AH d4-7)
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INFECTIOUS AND NONINFECTIOUS DISEASES OF CATTLE Animal Disease and Parasite Research Division, ARS

Problem. Losses from infectious and non-infectious diseases of cattle, other than those due to parasites, are estimated at approximately \$600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA AND COOPERATIVE PROGRAM

The <u>Department</u> has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The <u>Federal</u> scientific effort devoted to research in this area totals 57.7 professional man-years. This effort is divided among sub-headings as follows:

Brucellosis of Cattle 2.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the University of Minnesota, the University of Wisconsin, and with the Ohio Agricultural Experiment Station. A project on the immunizing effect of Brucella cell wall is in progress at the Hebrew University, Jerusalem, Israel, under a PL 480 Grant of funds equivalent to \$31,950.00 over a 3-year period.

Vibriosis of Cattle 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the New York State Veterinary College at Ithaca.

<u>Tuberculosis of Cattle</u> 6.6 at the National Animal Disease Laboratory, Ames, Iowa, and through two contracts with the Michigan State University at East Lansing.

Mucosal-Respiratory Disease-Complex 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Colorado State University at Fort Collins, the Agricultural Experiment Station, Purdue University at Lafayette, Indiana, and the Iowa State University, Ames.

Mastitis of Cattle 6.2 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Respiratory Disease of Cattle (Shipping Fever) 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Leptospirosis of Cattle 6.0 at the National Animal Disease Laboratory, Ames, Iowa.

Infertility in Cattle, other than Vibriosis and Trichomoniasis 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Epizootic Bovine Abortion 3.4 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the Agricultural Experiment Station at Ames.

Foot Rot (Infectious Pododermatitis) of Cattle 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Etiological, Cytological and Histochemical Studies of Pulmonary Adenomatosis in Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Immunization Against Bovine Leptospirosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Chemotherapy in Leptospirosis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Nature and Immunogenicity of Leptospiral Lipids 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Paratuberculosis of Cattle (Johne's Disease) 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Keratitis (Pink Eye) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

PROGRAM OF STATE EXPERIMENT STATIONS

The State experiment stations are active in conducting basic and applied research pertaining to the prevention, control and eradication of diseases of cattle. Objectives of these studies not only concern the health and wellbeing of animals but also reflect the increasing interest in the role of diseases of animals to the health of human beings. Research workers are concerned in delineating the cause of specific conditions, developing techniques for the improvement of diagnoses, finding new methods of increasing resistance to disease and/or decreasing the exposure to infectious agents.

Factors which affect the immune response in vaccinated calves and the development of new tests to increase the speed and accuracy by which brucellosis-infected animals can be detected are under investigation.

Cooperative regional studies among the Northeastern (NE-40, Pathology of Breeding Failure) and Southern States (S-30, Diseases of Reproduction) seek to determine the relation of infectious agents to poor reproductive performance and sterility in cattle. Antigenic variations in strains of the organism causing vibriosis are being studied to improve diagnostic techniques and to develop possible immunizing agents. The role of leptospira in infertility is being determined and detailed studies on the pathology produced by different serotypes of the organism are being elucidated.

Many of the North Central States are cooperating informally (NCR-37), Mucosal Disease; NCR-29, Shipping Fever) to determine the causes of bovine respiratory problems and to develop methods for control. Preventive vaccines are being developed and evaluated under laboratory and field conditions. The relation of infectious bovine rhinotracheitis to the respiratory disease complex is also being investigated.

Studies seek basic information pertaining to the cause of mastitis and the fundamental factors that influence resistance of individual cows. Prophylactic and therapeutic agents are being studied to evaluate their efficacy and milk residue properties.

Workers in many States are studying the interrelationships between various agents and factors associated with intestinal infections in cattle, particularly those causing severe losses in newborn calves.

Attempts are being made to clarify the cause of foot rot and infectious keratitis or pink eye. There is some evidence that viral agents may be responsible for these conditions.

Much attention (Regional Research Project, W-41, Urinary Calculi of Beef Cattle) is being given to possible factors which lead to the development of urinary calculi of cattle. Consideration is being given to the theory that an imbalance of certain nutritional elements may contribute to the development of the condition.

New diseases are being encountered constantly and diseases not previously encountered or not regarded as a problem, often become economically important enough to require intensive study. Other bovine disease problems being investigated currently include the various abnormalities, malignant lymphoma, tuberculosis, paratuberculosis, epizootic abortion, ketosis, parturient paresis, white muscle disease, aplastic anemia, enterotoxemia, etc.

The total State scientific effort devoted to diseases of cattle is 52.8 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Brucellosis of Cattle

Research work conducted at the National Animal Disease Laboratory (NADL), Ames, Iowa, was reported as follows:

- 1. Pathology: a) Two bulls, naturally infected with <u>Brucella abortus</u> were studied for 5 and 2 years, respectively. Serologic, bacteriologic and histopathologic examinations were correlated with the clinical signs of the disease. Seroagglutinin and semen plasma agglutinin titers persisted at diagnostic levels throughout the study, and <u>Brucella abortus</u> was consistently isolated from the semen of both bulls. At necropsy <u>Brucella abortus</u> was isolated from the testes, epididymides, seminal vesicles and the ampullae of the ductus deferens. Pathologic changes were observed throughout the genital tract. Granulomas, including sperm granulomas, were found in the epididymis of one bull.
- b) In two other bulls infected with brucellosis, the etiologic agent was Brucella abortus Strain 19. One bull, vaccinated at 6 months of age developed a bilateral orchitis within 10 days. Two months postvaccination a bilateral castration was required. A second bull was vaccinated at 5 months of age. Eight months later the seroagglutination test showed the bull had a titer of +50. The semen plasma test titer was +400. Brucella abortus Strain 19 was readily isolated from the ejaculate of each bull. At necropsy Strain 19 was isolated from the seminal vesicles, prostate, urethra and the epididymides. Pathologic alterations were primarily confined to the accessory genital organs and semen quality was not noticeably affected. The necessity and wisdom of employing the semen plasma agglutination test in addition to the blood serum tests was clearly indicated as a means of detecting potential spreaders of brucellosis.
- 2. Serology: a) Nonspecific agglutinins for Brucella were isolated from 7 cattle by techniques for absorption of serum by Brucella cells and differential ultracentrifugation. The agglutinins had high molecular weights with sedimentation coefficients varying from 13.8 to 16.6 Svedburg units. The isoelectric points of the purified agglutinins determined by microelectrophoresis correlated positively with the heat stability of the seroagglutinins. With one exception the heat stable (56 C, 18 hr.) agglutinins had an isoelectric point of pH 4.7, whereas the heat labile agglutinins had an isoelectric point of pH 4.3. The activity of the purified agglutinins ranged from 0.1 to 0.5 ug of protein per unit of agglutinin. The ultraviolet absorption maximums of the agglutinins fell into the range of typical proteins.
- b) These physicochemical studies were continued on Brucella agglutinins produced by heifers after vaccination with <u>Brucella abortus</u> Strain 19. One week after vaccination all agglutinins were of high molecular weight. In the second week postvaccination some low molecular weight seroagglutinins

were detected. The high molecular weight agglutinins reached a maximum concentration approximately 13 days postvaccination. Low molecular weight agglutinins reached a maximum at 28 to 42 days postvaccination. With few exceptions the fast sedimenting agglutinins predominated throughout the 91-day study. There was a positive correlation between the percentage of heat labile (65 C, 15 minutes) agglutinins and the percentage of fast sedimenting agglutinins. The percentage of agglutinins inactivated by mercaptoethanol was closely related to the percentage of fast sedimenting agglutinins.

c) A continuation of this research involved density gradient ultracentrifugation and heat stability (65 C, 15 minutes) studies on Brucella seroagglutinins of pregnant heifers artificially infected with virulent Brucella
abortus. During the first two weeks postexposure, all of the agglutinins
detected were high molecular weight type. Low molecular weight agglutinins
were first detected between the 15th and 29th days postexposure. As
infection progressed, the concentration of slow sedimenting (low molecular
weight) agglutinins became equal to and then exceeded that of the high
molecular weight agglutinins. As in the former studies there was a high
positive correlation between the percentage of heat labile agglutinins and
the percentage of high molecular weight agglutinins in each serum.

3. Immunology

Eighteen vaccinated and 5 nonvaccinated heifers in midgestation were exposed to virulent Brucella abortus strain 2308. Changes in their serum proteins were studied by paper electrophoresis for 29 weeks postexposure. In the serums of the heifers that became infected (4 vaccinated and 4 nonvaccinated), the relative percentage of gamma globulin over albumin was greater and persisted longer in the serums from infected nonvaccinated than in infected vaccinated heifers. Changes in the amount of gamma globulin roughly paralleled the changes in the seroagglutinin titers. Only minor changes occurred in the concentrations of albumin and globulin in the serums from 14 vaccinated and one nonvaccinated heifer that did not become infected. (Iowa) (ADP al-3(Rev.)

The University of Minnesota, under a cooperative agreement with the USDA, reported the studies during the past year were concerned with the study of physico-chemical characterization of antibodies for Brucella found in milk and serum of cattle and swine, and the development of methods to separate the several classes of antibodies for Brucella found in bovine milk.

(Minnesota) (ADP al-3(Rev.)

The University of Wisconsin, under a cooperative agreement with the USDA, reported work on a method of standardizing the complement-fixation (CF) test for bovine brucellosis, utilizing the Brucella antigen for the standard serum agglutination tube test. With the International Standard for anti-Brucella abortus serum, the method compared favorably in sensitivity with methods used in European laboratories.

Over 1400 serum samples from cattle in brucellosis problem herds in Wisconsin were examined by the standard serum agglutination tube test and the CF test. The CF test was a useful supplemental test for serums with suspect titers to the agglutination test. In several herds in which infection was recent, cattle developed CF titers before they became agglutination reactors.

(Wisconsin)

(ADP al-3(Rev.)

Research work was initiated at the Ohio Agricultural Experiment Station, Wooster, under a cooperative agreement with the USDA. The first phase of the study on early vaccination of calves against Brucellosis was completed. Calves 2 and 3-months of age, respectively, were tested by serological techniques and then vaccinated with Brucella abortus, Strain 19. (Ohio) (ADP al-3(Rev.)

Investigations on "The Immunizing Effects of Brucella cell wall" are in progress at the Hebrew University, Jerusalem, Israel, under a PL 480 Grant (AlO-ADP-6). The preliminary work, using experimental animals, has been mainly of a confirmatory nature. However, it appears encouraging. (Israel)

B. Vibriosis

Research conducted at the National Animal Disease Laboratory, Ames, Iowa, was reported as follows:

1. Reproductive Patterns of Vibrio fetus-infected Cattle. Work has been completed on a study of the breeding patterns of 31 female cattle bred for 1 to 4 successive calf crops to Vibrio fetus-infected bulls. Twenty-eight of these cows became infected at first service to an infected bull. One of the remaining 3 became infected at first service for her second calf; one became infected at second service to an infected bull, and the other did not become infected when bred for 2 pregnancies.

The average duration of infection with \underline{V} . $\underline{\text{fetus}}$ was 180 days, ranging from 14 to 313 days. All except 1 cow recovered spontaneously between gestation periods and remained free of infection until they were rebred, at which time 60% became reinfected. The exception was one heifer which remained infected during gestation and thereafter until necropsy, 66 days after calving, when \underline{V} . $\underline{\text{fetus}}$ was isolated from her uterus.

The cows required more services and more time from first service to pregnancy when first infected than did those which were reinfected; however, some reinfected cows also remained infected throughout subsequent gestation periods.

This study indicates that although immunity was not established with first infection, heifers artificially exposed with \underline{V} . \underline{fetus} at sexual maturity might be stimulated to produce resistance before service for their first calf crop and thus breed satisfactorily without significant lost time.

2. <u>Vibrio</u> Infection of the Digestive Organs of Cattle. Eighteen cattle were orally inoculated with broth suspensions of <u>Vibrio</u> fetus type 1, subtype 1, and type 2 to study the infectivity of each for the digestive organs. Six of 7 cattle fed type 2 became infected and shed the organism in their feces for variable periods. One cow remained infected for 4 weeks. She became reinfected after feeding thie type again and remained infected for 16 additional weeks. Another cow, infected for 6 weeks, did not become reinfected when inoculated a second time. Neither type 1 nor subtype 1 <u>V. fetus</u> infected any of 11 cows inoculated. At necropsy type 2 <u>V. fetus</u> was isolated from the duodenum, bile, bile duct, liver, and pancreatic duct of cattle up to 5 months after feeding.

It was apparent from this study that only type 2 \underline{V} . fetus infects the digestive organs of cattle and although type 1 and subtype 1 proliferated in the reproductive organs of cattle and caused repeat breeding, they are unable to live in the digestive organs. Type 2 has been considered an intestinal inhabitant which has the capacity to cause sporadic abortion in cattle and sheep.

3. Fluorescent Antibody Studies. Fluorescent antibody conjugates capable of producing fluorescence in <u>V</u>. fetus cells were prepared by several methods. It appears that the success of fluorescent staining varies with the method of serum fractionation employed. The performance of conjugates prepared from serum fractionated with ammonium sulfate was superior to that of other conjugates. Bright staining was observed more frequently with bovine serum conjugates than with conjugates of rabbit origin. While the staining of cell suspensions was rapid and simple, better results were obtained by staining smears. (Iowa) (ADP al-9(Rev.)

The New York Veterinary College, Cornell University at Ithaca, under a cooperative agreement with the USDA, continued research studies on diagnostic procedures for vibriosis. The following findings were reported:

- A. Incidence of vibriosis in an artificial insemination stud. From 1952 to July 1963, 12,644 semen samples from 432 bulls were cultured for Vibrio fetus. Analysis of the data indicated that 20.4 percent of all bulls examined were carriers of \underline{V} . fetus. There was a highly significant age effect on the incidence of \underline{V} . fetus. Of 233 bulls under 6 years of age, 4 (1.7 percent) were carriers, whereas 65 (46.7 percent) of 139 bulls 6 years of age or older were carriers.
- B. Diagnosis of vibriosis in the bull by use of fluorescent antibody technique. The objective of this project was to develop a fluorescent antibody technique for diagnosing vibriosis in bulls. Although culture techniques for recovering V. fetus from bulls have been improved during the last few years, limited numbers of culture attempts cannot be relied upon for detecting all carrier bulls. The use of virgin heifers as test animals is more accurate, but is too expensive for routine use.

The fluorescent antibody technique has been successfully adapted for diagnostic purposes by using a conjugate purified to eliminate most of the non-specific staining reactions and by concentrating the organisms in samples of sheath scrapings through centrifugation. On the basis of present results, it appears that this is a more sensitive method of diagnosis than the best culture methods and that it probably ranks with the heifer-mating test in its efficiency. (New York) (ADP al-9(Rev.)

C. Tuberculosis

Research was continued at the Michigan State University under two contracts with the USDA. Reports submitted are as follows:

(Contract No. 12-14-100-6852(45). Lipids extracted by ether-ethanol from 25 strains of mycobacteria were fractionated by absorption chromatography. Infrared spectra of the fractions were recorded. Type-specific lipid compounds were found in the extracts of human, bovine, avian and atypical strains.

Demoycoceronate of phthioceral was found in the lipids of two human strains (H37R_A and H37R_V) and one bovine strain (M. bovis Ravenel), mycoside B was isolated from M. bovis Ravenel but not from M. bovis B.C.G. Other type-specific lipids found were: mycoside A isolated from two photochromogens (P-4 and P-8), mycoside F from M. fortuitum, mycoside C from M. avium and 158 C-O (isolated from a bovine mesenteric lymph node), mycoside D from 71C-O (also from a bovine mesenteric lymph node) and mycoside C from strain P-31 and 12 organisms isolated from swine mesenteric lymph nodes and bovine body lymph nodes and Peyer's patches.

(Contract No. 12-14-100-7164(45). This contract was initiated during the reporting period. Experiments are in progress. Cattle were obtained which were not sensitive to tuberculin by caudal fold and cervical tests. The necessary facilities have been obtained to permit studies on chromatograph.

(Michigan)

Contract No. 12-14-100-5786(45), on the Role of Heat-Killed Mycobacteria and Feed Supplements of Animal Origin in Producing Tuberculin Hypersensitivity in Cattle, was completed, and the researchers at the Michigan State University submit the following summary of their findings:

Sixty nonpregnant predominantly Guernsey crossbred heifers were fed one of four different rations for 160 days to determine if any of the rations would induce delayed hypersensitivity in the animals as detected with 0.lcc mammalian tuberculin injected intradermally. The animals were obtained from a herd with no tuberculin reactors and had no detectable response to mammalian or avian tuberculins or johnin when tested in the caudal fold and cervical regions. They were maintained during the study in four isolated groups of 15 each. The control group was fed a ration in which the protein concentrate was soybean oil meal and the mineral concentrate was dicalcium phosphate. The second and third groups were fed the control ration to which daily was added 5 x 10^{8-9} heat-killed (121C moist heat for 30 minutes) Mycobacterium bovis and Mycobacterium avium, respectively. The fourth group was fed a ration in which the protein concentrate was meat and bone scrap and the mineral concentrate steamed bone meal.

Tuberculin tests using 0.1 cc mammalian tuberculin were performed on all animals at three different times. Some were tested at 20, 30 or 40 days, and all were tested at 100 and 160 days following the start of feeding the experimental rations. No animal was classified as a reactor at the official reading time.

(Michigan) (ADP al-13(Rev.)

D. Mucosal-Respiratory Disease-Complex of Cattle

Research studies were continued at the National Animal Disease Laboratory, Ames, Iowa. Reports submitted showed that calves inoculated with bovine viral diarrhea (BVD) viruses and soluble antigen, the complement-fixing (CF) antibodies appeared before serum-neutralizing (SN) antibodies and remained at high levels throughout the test period. A rapid rise in SN antibodies occurred after challenge with homologous virus with no apparent effect on CF antibody levels.

The CF antibody responses in calves infected with cytopathogenic NADL-MD and noncytopathogenic CG-1220 viruses were similar, whereas SN antibody responses indicated strain specificity by reciprocal cross-neutralization tests.

The CF antibody levels in 5 hog cholera (HC) antisera were assayed, using the soluble antigen of NADL-mucosal diarrhea-bovine virus diarrhea virus. No demonstrable SN antibodies were present in four HC antisera tested against NADL-MD virus, but a significant titer was present in the commercially prepared antiserum.

Virus was reisolated from animals infected with BVD viruses by buffy coat culture technique during 3 weeks' postinoculation, even when significant levels of CF and SN antibodies were present.

Noncytopathogenic (NCP) bovine viral diarrhea disease agents can be detected and titrated in tissue culture systems by a method employing immuno-fluorescence. Cytopathogenic (CP) and non-CP (NCP) viruses cross-react with fluorescein-conjugated serum globulins produced against either CP or NCP viruses, but the fluorescence is more intense in the homologous serum. Serum neutralization titers of sera against both CP and NCP groups were compared for both groups of viruses, and results of cross reactions were in agreement with results from immunofluorescence tests. (Iowa-NADL)

Colorado State University, Fort Collins, under a cooperative agreement with the USDA, reported that during the past year the serum neutralization titer of the cattle which were kept in the isolation units did not show lowering of titer. There were 3 lots of cattle with 4 animals per lot. One group was injected intratracheally, one intramuscularly, and the third lot served as control. There was no difference of titer between the two infected lots.

Pathological studies of infectious bovine rhinotracheitis in relation to abortion are being conducted using 35 virgin heifers that were negative to serological tests for this disease, brucellosis, and leptospirosis.

Progress is being made on this phase of the research. (Colorado)

Research work conducted at Purdue University, Lafayette, Indiana, under a cooperative agreement with the USDA, was a continuation of tissue culture, fluorescent antibody, and serological investigations.

Sporadic cases of the mucosal disease complex continue to occue in Indiana. The apparent incidence of this disease complex has not changed from previous years.

Cytochemical and cytological studies on the growth of Oregon C24 virus in tissue culture were made. The application of the acridine orange (AO) staining procedure to infected lamb thyroid cultures gave evidence that C24 virus is of the RNA type. Furthermore, AO and phase microscopic studies suggest that replication takes place in the cytoplasm of infected cells. The data derived from these and other growth studies will be utilized in applying fluorescent antibody procedures for detection of virus diarrheamucosal disease agents in clinical specimens and tissue culture systems.

On initial passage C24 virus was capable of producing cytopathic changes in cultures of bovine and ovine kidney, testicle and thyroid tissues. In an explant-type culture system employing lamb kidney tissue, cytoplasmic inclusion-like lesions were observed. The development of cytoplasmic inclusions and the general cytopathic effects of virus were inhibited by specific immune serum. Further study is needed to determine the specificity of the cytoplasmic lesions observed.

Two virus isolations made from field cases of "mucosal disease" were grown on bovine embryonic kidney and lamb thyroid cells. Serums from Specific-Pathogen-Free calves recovered from experimental infection with the two field isolates neutralized Oregon C24 in tissue culture tests. The new virus isolates appear to be immunologically and serologically related to other virus diarrhea-mucosal disease viruses.

The specific-pathogen-free (SPF) cattle herd continues to be relatively free of important pathogens. The reproductive efficiency of the herd is normal and about 24 calves will be available for research during the next twelve months. (Indiana)

At the Iowa State University during the past year, research results have pointed to the fact that both viral diarrhea and infectious bovine rhinotracheitis may elicit a clinical and pathological syndrome which is indistinguishable. They have verified this fact by fluorescent antibody staining of viral antigen associated with Herpes-virus-induced lesions. Results further indicate that the entire group of enteroviruses may be excluded from the viral diarrhea problem in cattle, but play an important role in enteric problems of young calves. (Iowa State Univ.)(ADP al-14C(R)

E. Mastitis of Cattle

The research studies at the National Animal Disease Laboratory, Ames, Iowa, pertained to the following:

1. Three cultures of group A hemolytic streptococci have been serially subcultured for an extended period (100 or more serial transfers) in a peptide- and protein-free medium. In 24-48 hours incubation at 37°C, luxuriant growth was obtained with complete removal of 1 percent glucose and quantitative fermentation to lactic acid. Optical densities of cultures were 0.40 - 0.50. An amino acid assay medium, modified by addition of small amounts of glutamine, ammonium acetate and 0.1 M phosphate, pH 7, was used. In this medium high concentrations of glutamic acid or glutamine were required and biotin was stimulatory to growth. Biotin could be partially replaced with NaHCO₂. Maximal growth was obtained with NaHCO₃ when biotin was present and aspartic acid and asparagine were omitted from the medium.

2. Three strains of Streptococcus pyogenes, Richards (type 3), N19 (type 19) and S43 (type 6), after repeated subculturing in a chemically defined medium (100 or more times), were each tested for the group-specific and type-specific antigens by the precipitin test. On three separate tests for each culture, the group A antigen ("c" polysaccharide) was present, but the type-specific antigen (M-protein) was absent. The same strains grown on a chemically defined medium containing reduced ovalbumin showed no loss of M protein. (Iowa - NADL)

The University of California, Davis, under a cooperative agreement with the USDA, reported that the results of several years of investigation indicate that coliform mastitis is a disease of the normal lactating mammary gland. It was concluded that the relative infrequency of occurrence of coliform mastitis, in commercial dairy herds, is due to the presence of a leukocyte barrier in lactating glands of older cows. This leukocyte barrier is in response to the stress on mammary tissues of modern methods of mechanical milking and bacterial infection with common udder pathogens.

To further substantiate the role of the leukocyte in controlling multiplication of coliform bacteria within the udder, an attempt was made during the current year to delay the infiltration of leukocytes into exposed mammary glands. To this end, corticosteroids, both intramammarily and systemically were employed. Selection of corticosteroids for this purpose was based on the claimed ability to inhibit diapedesis of leukocytes into foci of developing inflammation. The corticoid employed was 9% fluoroprednisolone acetate (Upjohn) at dose levels of 50 mg. to 1,000 mg. per cow. Such doses were greatly in excess of the quantities commonly incorporated in antibiotic preparations for therapeutic treatment of mastitic glands. Administration of the corticoid prior to and simultaneously with coliform bacteria failed to delay or reduce the magnitude of the leukocytic infiltration. Despite the mobilization of circulating neutrophil leukocytes to levels up to 6 times normal following systemic application of the corticoid, the leukocytic activity within the mammary gland exposed to coliform bacteria was not enhanced. Escherichia coli and Aerobacter aerogenes are not considered true pathogenic bacteria. Clinical disease is produced by the release of endotoxin when the bacterial cells of a massive population are destroyed by leukocytic action.

In order to determine if a leukocyte barrier can exist for recognized pathogens of the mastitis complex, attention was directed toward Streptococcus agalactiae. Through natural selection this organism has become an obligatory parasite of the mammary gland. Its potential for establishment of an enduring infection within the mammary gland is well known. Four lactating heifers and 5 older cows, none of which had any previous exposure to Str. agalactiae were available. Pre-existing leukocytic infiltration into mammary quarters were of natural or experimental origin for investigation of the leukocyte barrier. An adequate number of quarters secreting cell-free milk was available for controls. Among 16 glands serving as control and receiving a single exposure to Str. agalactiae at levels of

5 to 600 colony-forming units, infections were established in 6 glands (37%); among 7 control glands exposed 3 times at 12-hour intervals to between 6 and 400 Str. agalactiae, the infection rate was 50 percent. Thus, it was established that small numbers of Str. agalactiae are potentially capable of establishing infection in essentially normal lactitating quarters.

Failure of <u>Str. agalactiae</u> to establish itself within an exposed lactating mammary quarter may be in consequence of one or more or a combination of the following:

- 1) Pre-existing leukocytic infiltration at levels serving as a barrier per se to Str. agalactiae multiplication.
- 2) Capability of a gland to infiltrate millions of leukocytes within the first few hours after introduction of <u>Str. agalactiae</u>. Glands previously injured but having returned to low infiltrating cell numbers appear to be uniquely capable of immediate mobilization of large numbers of leukocytes.
- Participation of humoral factors from infection of opposite quarters with <u>Str. agalactiae</u> and, perhaps, other streptococci.

Experiments on the endotoxin of \underline{A} . aerogenes confirmed a previous hypothesis that the clinical signs of \underline{A} . aerogenes peracute mastitis were probably referable to the release of endotoxin following lysis of acteria by the inflammatory exudate. Endotoxin at levels of from 0.2 mg to 20.0 mg produced the same array of signs and symptoms as those seen following unlimited growth of \underline{A} . aerogenes. The endotoxin had all the characteristics of endotoxin from R variants of gram negative bacteria. Trials in cats and a horse showed cats to be rather resistant to the endotoxin, whereas one horse died within 9 hours following administration of a safe dose calculated from mouse inoculation data.

The probable identity of the protein "X" with paper electrophoretic mobility intermediate between a lactal bumin and immune globulin that appears in whey following agalactia of mastitis or in early dry cow secretion was confirmed. The protein when isolated by preparative electrophoresis does not have the same mobility in the isolated state but behaves as immune globulin. The isolation procedure is considered to be sufficiently mild that denaturation was not involved.

(California) (ADP al-15(Rev.)

F. Respiratory Diseases of Cattle (Shipping Fever)

Research investigations conducted at the National Animal Disease Laboratory, Ames, revealed that smooth <u>Pasteurella haemolytica</u>, after rapid growth in statically incubated broth cultures, decreased in numbers rapidly, and were replaced by nonsmooth variants. Upon continued incubation, smooth cells again predominated. The two phenotypes were alike in general biochemical characteristics, but differed in virulence for mice. The presence of non-

smooth cells in mixed cultures limited the growth of smooth cells. The inhibition of smooth cells correlated with the establishment of definite population densities, and the critical factor was limitation of oxygen in the cultural medium. Selective inhibition did not occur in aerated cultures, but was more pronounced in cultures grown under reduced air pressure. Selective death of smooth cells on slant cultures held at 5°C, and preferential growth of nonsmooth cells, plus death of smooth cells at room temperature, accounted for population changes in stored cultures.

Bovine parainfluenza-3 (PIV-3) virus was isolated from nasal mucus of cattle with signs of shipping fever by amniotic inoculation of 14-day-old embryonated hen's eggs. Virus isolated in this manner could not be demonstrated in the amniotic fluid, but after 3 passages in the amnion could usually be recognized by agglutination of guinea pig erythrocytes. Primary virus isolates could, however, be recognized by inoculation of embryonic bovine kidney (EBK) cells. Multiplication of PIV-3 virus in the embryonated hen's egg did not result in death of the embryo.

Virus isolations from diluted specimens suggested that the chick embryo is more susceptible to PIV-3 virus infection than are tissue cultures of EBK cells. Egg inoculation also permitted the selection of PIV-3 virus in the presence of infectious bovine rhinotracheitis virus which was demonstrated in several samples. Attempts to adapt virus isolated in the amnion to the allantoic cavity of younger embryos were not successful. (Iowa-NADL)

(ADP al-17)

G. Epizootic Bovine Abortion

The University of California, under a cooperative agreement with the USDA, reported the following:

- 1. Studies to be terminated this year indicate conclusively that vaccination with a modified live virus vaccine prepared from an agent (Miyagawanella felis) related to the virus of epizootic bovine abortion (EBA) is ineffective in preventing abortion due to the EBA virus. The current approach to preventive immunization is by means of a vaccine consisting of an attenuated strain of EBA virus, given when heifers are six months of age and repeated just prior to breeding. Attempts to attenuate the virus are currently in progress.
- 2. In view of indications that EBA is a venereally-transmitted infection, studies designed to determine the validity of this observation are under way. Should this mode of transmission be proved, artificial insemination is regarded as the ultimate solution to the problem of prevention and control.
- 3. Progress in serological studies relative to the EBA virus has been made.

- 4. Preliminary findings suggest that abortion due to the virus of infectious bovine rhinotracheitis (IBR) is restricted to those strains of virus which have acquired an enhanced invasiveness for the blood stream of cattle. This property appears to have been acquired fairly recently as studies of early respiratory isolates of the virus indicate that such strains lack this characteristic.
- 5. The virus of enzootic abortion of ewes (EAE) has been isolated in ewes in California and Oregon. This represents the first isolation of this virus outside the enzootic areas of Montana, Idaho, and Utah. (California)

 (ADP al-21)

H. Immunization Against Bovine Leptospirosis

The National Animal Disease Laboratory, Ames, Iowa, reports that Leptospira pomona and 13 other leptospiral serotypes, were subcultured at weekly intervals for 2 years in a medium primarily composed of Oleic Albumin Complex and NH_LCl. The albumin functioned as a detoxifier of oleic acid and as a source of nitrogen because continuous subculture was possible without adding NH_LCl, but at a markedly reduced level of growth. Added vitamin B₁₂ was required for growth of representative members of each serotype studied. Additions of NaCl stimulated growth.

A commercial complex of bovine albumin and oleic acid (OAC), which replaced whole rabbit serum in leptospiral medium, was fractionated. The growth—supporting function of each fraction was studied, and the fractions were replaced with specific nutrients. Basal medium supplemented with bovine albumin and sodium oleate or Tween 80 supported good growth of 14 leptospiral serotypes through indefinite subcultures with undiminished growth and unaltered antigenicity.

Oleic albumin complex was extracted with ether. The ether extract, when recombined with extracted OAC, supported good growth. Alkalinized oleic acid, sodium oleate, or Tween 80 satisfactorily supplemented several albumins of bovine origin. Adding lipid to spent medium restored its growth-supporting capability. If Tween 80 was used, 0.5% albumin was adequate for cultivation of Leptospira pomona.

Leptospira grippotyphosa was isolated from the urine of a cow 7 days after abortion. The isolant grew poorly in Stuart's liquid medium and Fletcher's semisolid medium. Experimental semisolid and liquid media, containing bovine albumin fraction V and Tween-80, proved valuable as isolation and growth media. Gerbils and hamsters were more susceptible than guinea pigs and white mice to the newly isolated organism. Serological evidence indicates that L. grippotyphosa is widely distributed in Illinois cattle and swine. (Iowa-NADL) (ADP al-25)

I. Chemotherapy in Leptospirosis

Investigations at the National Animal Disease Laboratory, Ames, Iowa, have determined the effects of certain antibiotics and polylysine on leptospirae in synthetic medium and medium supplemented with rabbit serum. No differences in sensitivity to antibiotics and polylysine were found among cultures of Leptospira pomona, L. canicola, L. autumnalis, and L. grippotyphosa in synthetic medium. All the antibiotics tested were leptospirastatic in low concentrations. Tylocin and erythromycin were effective in the lowest concentration (0.025 µg/ml) and sterilized cultures the quickest (72 to 96 hours); chlortetracycline and oxytetracycline (0.5 µg/ml) prevented multiplication but failed to sterilize cultures in 10 days. Little or no leptospiral immobilization was observed in cultures containing bacteriostatic levels of antibiotics; most antibiotics lysed leptospirae at ten times the leptospirastatic concentration. In Stuart's medium containing 10% rabbit serum, penicillin and oxytetracycline were two and four times less effective than in synthetic medium, respectively. (Iowa-NADL) (ADP al-26)

J. Nature and Immunogenicity of Leptospiral Lipids

Research workers at the National Animal Disease Laboratory, Ames, Iowa, reported that Leptospira canicola cells were grown in a chemically characterized medium containing Tween 80. Lipid extracted with chloroform: methanol (2:1) from washed, lyophilized cells was equivalent to 16% of the dry cell weight. Approximately 50% of this lipid was present in the phospholipid fraction.

Fatty acids from whole cells were tentatively identified by gas-liquid chromatography of the methyl esters using diethylene glycol succinate and Apiezon L as liquid phases. Unsaturated esters were removed as mercuric acetate adducts.

Of the dialyzable lipid, octadecenoic acid was the major acid accounting for 47% by weight of the fatty acids. Hexadecanoic acid accounted for 19% of the fatty acids. The next largest component (9%) was an unidentified, unsaturated fatty acid with a carbon number of 15.25 on the DEGS polyester column. The other acids listed in descending order of abundance were: an unidentified saturated acid with the same retention volume as a 17-carbon branched-chain acid, hexadecenoic acid, octadecaienoic acid, tetradecenoic acid, an unsaturated acid with a carbon number of 12.75, octadecanoic acid and traces of octanoic, tetradecanoic acid and several other unidentified acids. Acids with retention volumes corresponding to 17 or 19-carbon cyclopropane fatty acids were not noted. (Iowa-NADL) (ADP al-27)

K. Paratuberculosis (Johne's Disease) of Cattle

The National Animal Disease Laboratory, Ames, Iowa, reported that experiments were conducted to find a combination of decontaminant and medium that would be more satisfactory for the primary cultivation of Mycobacterium paratuberculosis. Sodium hydroxide, sodium hypochlorite, phenol, and benzalkonium chloride (Zephiran) were compared as decontaminants, and specimens treated with these agents were cultured on lymph-node-egg-yolk medium and modified Herrold's medium (an egg-yolk-agar medium containing mycobactin). The most satisfactory combination was decontamination with benzalkonium chloride, followed by inoculation onto modified Herrold's medium. This technique allowed the demonstration of Myco. paratuberculosis in tissues in which the organisms were present in such small numbers that they could not be found by microscopic examination.

Blood samples were obtained periodically for complement-fixation tests from all cattle in a herd of 161-195 where Johne's disease has been an economic problem. Selected tissues of all cattle removed from the herd were examined for Mycobacterium paratuberculosis after slaughter.

Observations were made on 93 cattle eliminated from the herd during a 5-year study. Fifty-four cattle had titers of 1:32 or more; 12 of these developed clinical evidence of Johne's disease, and 23, including the aforementioned 12, harbored M. paratuberculosis. Thirteen of the remaining 39 cattle with serum titers of 1:16 or less harbored the bacillus at slaughter, and 3 of these had developed clinical evidence of Johne's disease. Forty cattle were tested 6 times in 2-1/2 years, a total of 240 tests; titers remained constant or changed only 1 dilution in 185 instances. It increased 2 dilutions in 14 instances, 3 dilutions in 6 instances, 4 dilutions in 5 instances, and 5 dilutions in 1 instance. The titer decreased 2 dilutions in 24 instances, 3 dilutions in 4 instances and 5 dilutions in 1 instance. Six calves, a few days to 2 months old, had titers of 1:32. Titers disappeared within 6 months. (Iowa-NADL) (ADP al-35)

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FOOT-AND-MOUTH AND OTHER EXOTIC INFECTIOUS DISEASES OF CATTLE Animal Disease and Parasite Research Division, ARS

Problem. The Congress in 1948 authorized establishment of a laboratory in the United States for research on foot-and-mouth and other exotic animal diseases. The law required that the laboratory and related facilities for research and study be located on a coastal island separated from the mainland by deep, navigable waters. Plum Island was selected as the site for the laboratory on July 28, 1952. The Plum Island Animal Disease Laboratory as a U. S. Department of Agriculture venture came into existence on July 1, 1954, and since that time this laboratory has been responsible for protecting the nation's livestock industry against animal diseases of foreign origin. Foot-and-mouth disease has visited the United States on 9 occasions and each time has been eradicated. The last outbreak of foot-and-mouth disease was in 1929. Contagious bovine pleuropneumonia was eradicated in the 1880's and has not recurred since. Success in keeping these exotic animal diseases out of the United States has been due to a number of factors and a continuing vigilance by U. S. Department of Agriculture personnel.

The establishment of the Plum Island Animal Disease Laboratory and its continuing research program on exotic animal diseases has provided a laboratory in the United States where research on animal disease foreign to our soils is carried out. As new information is developed at the laboratory, it is made available to those agencies in the Department responsible for keeping out livestock animal diseases which do not occur in this country. Foot-and-mouth disease is capable of reducing our overall productivity by 25% in areas where it might become established. The disease exists in all large land areas of the world with the exception of Central and North America, Australia, and New Zealand.

Rinderpest, a disease of cattle, continues to be a serious disease problem in Africa and Asia. This disease is capable of killing 90% or more of the cattle exposed to it. Other diseases for which the laboratory is responsible include contagious bovine pleuropneumonia, Rift Valley fever, East Coast fever, and lumpy skin disease. All of these diseases continue to cause severe losses in other parts of the world. The possibilities of entry of these diseases in the United States continues, primarily because of the progressively increasing scope, speed, and extent of modern international transportation. Information developed at the Plum Island Animal Disease Laboratory is applied to the protection of the nation's livestock against foreign animal diseases.

The research continues to develop and maintain a competence for diagnosis of exotic animal diseases. Fundamental research is being conducted on biological, chemical, and physical properties of the infective agents that may be useful in prevention, control, and eradication of these diseases.

USDA AND COOPERATIVE PROGRAM

The <u>Department</u> at its Plum Island Animal Disease Laboratory has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists engaged in basic and applied research in this problem area. All of this research is conducted at the Plum Island Animal Disease Laboratory, Greenport, New York, except for supplemental field studies on foot-and-mouth disease vaccines which is conducted cooperatively in the Netherlands. The Department is also engaged in research under terms of an Interagency Agreement with the Assistance In Development Program, U. S. State Department, in Kenya, on contagious bovine pleuropneumonia.

The <u>Federal</u> scientific effort devoted to research in this area conducted solely at the Plum Island Animal Disease Laboratory, totals 28.5 professional man-years. This effort is divided among sub-headings as follows:

Histopathology -- foot-and-mouth and other exotic diseases 1.0

Fluorescent antibody technique to locate viruses 1.0

Studies on foot-and-mouth disease virus 2.0

Determine mechanism of antibody formation 0.5

Immune response of cattle to types and sub-types of foot-and-mouth disease virus 1.0

Quantity production of foot-and-mouth disease virus 2.0

Microcinematography of cellular reaction of infected cells 0.5

Establishment and characterization of cell lines and cell strains 1.0

Mechanism of the interaction between foot-and-mouth disease virus molecules and host cells 2.0

Genetic biochemistry of foot-and-mouth disease virus 1.0

Effects of chemical and physical environment on foot-and-mouth disease virus 1.0

Bulk Freeze Drying of foot-and-mouth disease virus vaccine and antiserum 1.0

Investigations of Rinderpest in Cattle 2.5

Survival and Transmission of Foot-and-Mouth Disease Virus in Semen 1.0

- Identification, purification and chemical and physical characterization of foot-and-mouth disease virus and other exotic animal viruses 2.0
- Immuno-chemical investigations of foot-and-mouth disease virus 1.0
- Attenuation of representative types of foot-and-mouth disease virus 1.0
- Survival and inactivation of foot-and-mouth disease virus in meat and meat by-products 1.0
- Biological mechanism of natural resistance and susceptibility to footmouth disease virus 1.0
- Biological alteration of foot-and-mouth disease virus from continual residence in cell cultures 1.0
- Morphological aspects of virus-cell relationships 1.0
- Diagnostic and immunizing procedures for contagious bovine pleuropneumonia 3.0

Work was continued under a PL 480 grant to the Instituto Biologica, Sao Paulo, Brazil for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus, and experimental field vaccination.

Under a PL 480 grant to the Ministry of Agriculture, Laboratories of Footand Mouth Disease and Tissue Culture, Etlik, Turkey, research is under way on "Studies of Various Indigenous Types of Foot-and-Mouth Disease Virus, and the Production of a Vaccine for the Control of Foot-and-Mouth Disease in Turkey."

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Histopathology -- Foot-and-Mouth and Other Exotic Diseases

It was demonstrated that the initial mucosal and epithelial lesions of both foot-and-mouth disease and vesicular stomatitis consist of circumscribed, degenerated areas of epithelial tissue. The characteristic vesicles develop from these initial lesions; but vesiculation may not and does not always occur. This knowledge is useful in clinical recognition of the two diseases.

A histopathological survey of cattle received at Plum Island during a period of one year revealed a high incidence of sub-clinical (renal) leptospirosis. Clinical recognition of the disease had never been made on the farm where the cattle were raised. The combined microscopical and serological data indicated the probability of infection with more than one serotype. Information obtained was useful in developing concepts of the character of the disease in cattle.

Microscopic pathology of foot-and-mouth disease in pregnant and lactating mice was described.

Lesions in guinea pigs resulting from estrogen contamination of pelleted feed were described. (ADP a8-1(Rev.)

B. Fluorescent Antibody Technique to Locate Viruses

A fluorescent antibody technique for antigens and/or antibodies related to foot-and-mouth disease was developed. Commercial reagents (except the immune sera) were used. Serums from cattle infected with any of the seven types of foot-and-mouth disease (FMD) consistently gave positive or (in only two instances) suspicious fluorescent antibody reactions. Serums from cattle with a lesser degree of immunity than that conferred by frank infection with virulent virus associated with lesion development, gave negative fluorescent antibody reactions. The technique appears to be useful for detecting animals convalescent from foot-and-mouth disease (from one week to as long as 2 years following infection), but did not distinguish between types of infection.

(ADP a8-2(Rev.)

C. Studies on Foot-and-Mouth Disease Virus

Foot-and-mouth disease virus (FMDV), type A, strain 119, was produced in baby hamster kidney cell cultures and treated with acetylethyleneimine (AEI). The AEI-treated preparation was used to prepare a colloidal aluminum gel (Alhydrogel) vaccine and an oil emulsified vaccine. In the animals vaccinated with oil emulsion vaccine, the neutralizing and complement-fixing antibodies were maintained at levels 30 to 200 times greater than in the animals that received the Alhydrogel vaccine and similarly to that found in animals infected with FMDV. The 19S type antibody was difficult to demonstrate in both groups and did not approach the high level produced in animals undergoing actual infection.

The oil emulsified vaccine was superior to the Alhydrogel vaccine in terms of inducing higher levels of antibody which persisted for a relatively longer period of time.

An understanding of the degree and duration of immunity conferred by FMDV as well as a knowledge of practical methods for evaluating immunity is essential to properly plan and implement field vaccination programs. Investigations of this type require the use of large numbers of cattle over long periods of time and can best be carried out in an area where FMD vaccination is routinely practised. Such studies are being pursued in Holland in cooperation with the Netherlands Ministry of Agriculture. A number of herds consisting of approximately 400 cattle vaccinated and held under field conditions, are included in this study. Vaccinated cattle presented for slaughter at the Amsterdam abattoir are also available.

Serum antibody levels against types 0 and A FMDV remained high for over two years in most cattle which had received two or more annual vaccinations. An average of 80% of the animals which had experienced three or more annual field vaccinations showed resistance 16-48 months later when exposed to virulent FMDV. In general, a good correlation was observed between serum antibody level and resistance to infection, however, indications are that this relationship may vary somewhat depending on the interval of time elapsing between vaccination and exposure to the virus. (ADP a8-8(Rev.)

D. Determine Mechanism of Antibody Formation

The serologic, physical and chemical characteristics of antibodies produced by guinea pigs and cattle following infection with FMDV or inoculated with inactivated virus preparations, were investigated. The first appearing antibodies were macroglobulins having several different characteristics than the late appearing antibody. The time course of appearance of these two antibody types in the intact animal were studied to provide a better basis for subsequent cellular level experimentation. (ADP a8-10(Rev.)

E. Immune Response of Cattle to Types and Sub-Types of Foot-and-Mouth Disease Virus

Antibody formed in guinea pigs against the noninfective component of FMDV is specific for the noninfectious component, possesses no detectable virus neutralizing activity, and with the limited number of strains tested, appears to be type specific.

Assays of virus neutralizing antibody by tissue culture methods consistently resulted in lower values than those obtained when suckling mice were used as the assay system. Factors influencing this difference were the time and temperature of incubation of the antibody-virus mixture and the presence of a "persistent" fraction.

(ADP a8-11(Rev.)

F. Quantity Production of Foot-and-Mouth Disease Virus

A simplified method for preparing bovine calf kidney cells for growth on glass has been developed. Yields of 60 ml. of packed cells are routinely obtained from 100 Gm. of cortical tissue. One thousand or more plaqueforming units (PFU) of FMDV per cell were obtained from cell cultures prepared by this method for growth and assay of the virus.

Although dispersed cells were centrifuged to remove trypsin before preparing cultures, satisfactory cultures were prepared from dispersed cells which had not been centrifuged.

Application of information developed during the year on the effect of various chemical and physical factors on cell susceptibility has resulted in preparation of primary bovine calf kidney cells with increased susceptibility to infection with FMDV. These cells were used for plaque assay

of all seven types of FMDV isolated directly from animals. Plaque assay titers were similar to those obtained in adult steers and suckling mice, demonstrating the utility of these cells for use in detection and assay of field samples of FMDV. Increased yields of FMDV from early passage in these cells reduces or eliminates the necessity of adapting FMDV to tissue culture. This finding may have important application to vaccine production.

For a 7-month period considerable effort was diverted to experiments on detection and assay of virus from lymph nodes of cattle inoculated in Argentina with FMDV. (ADP a8-12(Rev.)

G. Microcinematography of Cellular Reaction of Infected Cells

Development of a microcinematographic technique that is practical for use under animal disease quarantine conditions was accomplished at PIADL.

First indications of culture survival after infection with FMDV were observed in connection with work on this project. This information initiated a line of investigation carried on under Line Project ADP a8-30.

Observations were recorded on the cytopathic effect of FMDV and rinderpest on a variety of cultured cells. (ADP a8-13(Rev.)

H. Establishment and Characterization of Cell Lines and Cell Strains

A lamb testis cell line developed at PIADL was used in determining the neutralizing activity of serums containing antibody against mucosal disease virus. Cells of the lamb testis line have withstood freezing and storage at about -70C for three and one-half years. (ADP a8-14(Rev.)

I. Immuno-Chemical Investigations of Foot-and-Mouth Disease

The physical-chemical characteristics of antibodies produced by guinea pigs and cattle in response to infection with FMDV were investigated. Animals responded with the early appearance of a macroglobulin type antibody (19S) that disappeared by about the 30th day following infection. Within a few days following the appearance of the macroglobulin antibody (19S), antibody of a smaller size (7S) was demonstrated and this antibody persisted at high levels over an extended period of time. Various other serologic, physical and chemical differences were also found for these two types of antibody. Cattle immunized with inactivated virus preparations showed some qualitative and quantitative differences in the time course of appearance of these antibodies when compared to infected animals. Inactivated virus emulsified in oil induced higher antibody levels that persisted for a longer time than when the inactivated virus was adsorbed to aluminum hydroxide gel.

(ADP a8-26)

J. Attenuation of Representative Types of Foot-and-Mouth Disease Virus

Concentrations of glycidaldehyde (GDA) as low as 0.005%, inactivated high titered FMDV of guinea pig vesicular fluid origin. A 0.05% concentration was effective in less than 30 minutes. No GDA-resistant virus particles were detected during the inactivation studies. Inactivation rate was directly related to the ambient temperature up to 42°C and virtually disappeared at 5°C.

Glycidaldehyde compared very favorably with acetylethyleneimine as an inactivant for $FMDV_{\bullet}$

The neutralization of GDA by sodium thiosulfate is not instantaneous and concentrations as high as 10% failed to neutralize the virucidal activity of GDA.

The reactivity of GDA-treated virus with specific immune serum was essentially the same as for control preparations. CF titers as high as 1:240 were consistent in both GDA-treated and untreated antigen.

The precipitin pattern, using the agar-gel technique, did not indicate virus breakdown. Precipitin antibody was detected 7 days postinoculation from a single injection of GDA-inactivated virus. (ADP a8-27)

K. Survival and Inactivation of Foot-and-Mouth Disease Virus in Meat and Meat By-Products

A total of 42 Argentine cattle, repeatedly vaccinated against FMD with trivalent vaccines (Types A, O and C), were selected for this experiment and tested in 3 groups. Each group of 14 vaccinated and 5 unvaccinated (control) mature cattle was infected with one of the 3 types (either A, O or C) FMDV. They were slaughtered 30 to 34 hours after infection. One lymph node was removed from one hind leg of each steer at slaughter. The corresponding lymph node from the other leg was obtained after ripening (chilling) the whole carcasses for 3 days. Several other lymph nodes were packed between meat chunks in wet salt-cure in barrels.

All lymph node samples and barrels of cured meat were shipped under strict safety precautions to the Plum Island Animal Disease Laboratory, U.S.A., and tested for the presence of virus using the most sensitive methods known. FMDV was detected in all fresh and all but one ripened lymph node sample from 15 unvaccinated (control) cattle. Virus also was found in 4 of 15 salt-cured samples held at 4C, 38-39 days. After infection, 2 of 42 vaccinated Argentine cattle developed tongue lesions containing virulent virus. Footand mouth disease virus was isolated from a lymph node in 1 of these 42 unvaccinated cattle.

In view of these results, it was concluded that FMDV may be present in the lymphatic system of vaccinated, and subsequently infected cattle. Presently

available vaccination methods do not prevent the dissemination of FMDV through meat.

Using 7 known types of FMDV, A, O, C, SAT-1, SAT-2, SAT-3, and Asia 1, it was shown that bovine lymph nodes contain virus as early as 12 hours and as long as 15 days after inoculation. While considerable amounts of FMDV may be present in lymph nodes, it might be difficult to diagnose the disease by routine inspection procedures at the preclinical and convalescent stages of infection. Cattle slaughtered during the course of inapparent infection may propagate FMDV through animal products.

Foot-and-mouth disease virus was detected in joints of infected cattle and survived in synovial fluid of infected carcasses for 19 days when stored at 4C. Virus remained infectious for several weeks in joints stored successively at chilling, freezing, and thawing temperatures.

Foot-and-mouth disease virus was remarkably stable in blood and infected or contaminated animal tissues which had been spread on materials used to package meat (wood, paper, metal). In several tests, the virus survived 48 days in blood spread on a can and stored at 4C. These preliminary results indicated that meat shipping containers may play a significant role in disseminating FMDV.

(ADP a8-28)

L. Biological Mechanism of Natural Resistance and Susceptibility to Footand-Mouth Disease Virus

Infant mice are highly susceptible to FMDV but develop a pronounced resistance as they mature. However, during the period of late pregnancy to about two weeks postpartum, 50-70% of female mice will succumb to FMDV. Factors which might be related to the mechanisms of these responses have been investigated:

- 1) Suspensions of minced kidneys from individual l-week-old mice, which are uniformly susceptible to FMDV, produced virus quickly and to high titers with only slight variation between preparations. In cells from less susceptible 5-week-old mice, however, there was considerable variation between preparations in time when multiplication began, time of peak titer, and amount of virus produced.
- 2) Mother mice with litters reacted with much less sensitivity after passive transfer of serum from mice sensitized with bovine serum than did similar mothers following removal of litters or nonmated controls. Similarly, serum from sensitized mother mice with litters produced less sensitivity in nonmated female mice than did sera from mother mice without litters or from nonmated control mice.

3) After being subcultured eight times cells originating from calf kidneys were less susceptible to FMDV than cells from the primary culture. Experiments indicate that selection of resistant cells occurs during subcultivation and that many serial passages of FMDV in primary cells results in the selection of virus with more virulence for the subcultured cells.

(ADP a8-29)

M. Biological Alterations of Foot-and-Mouth Disease Virus from Continual Residence in Cell Cultures

Type C₂ Rio foot-and-mouth disease virus and several line of type A-119 have been established in chronic residence on cultured bovine kidney cells. In all instances, a reduction in virulence has been effected. Total virus in work harvests from calf kidney cultures ranges from 10^{6.5}to 10^{8.6} TCID The amount of virulent virus in the various harvests (as determined by intralingual inoculations of cattle) ranges from 10² bovine ID to 10⁵ bovine ID depending on time in chronic residence and degree of modification. Indications are that the loss in virulence results from virus selection rather than a mutation or genetic change.

Conventional rapid serial passage of one line of modified virus 14 times in calf kidney cultures resulted in an increase of one log of residual virulent virus in the final harvest $(10^{2\cdot0}\text{to }10^{3\cdot1}\text{ ID}_{50})$ and partial restoration of the plaque forming ability $(10^{5\cdot7}$ as compared with $10^{7\cdot5}$ TCID₅₀). However the plaques were very small compared with average plaque produced by normal virus.

Virus populations with reduced virulence for cattle also had reduced virulence, but significant antigenicity for sheep.

Resistant cell lines developed from cultures cured of chronic infection may have value for use in more rapid selection of avirulent virus populations.

(ADP a8-30)

N. Morphological Aspects of Virus-Cell Relationships

Work was begun on the initial phase of the project, namely, development of primary or permanent cell lines which would react slowly to FMDV, or replicate the virus without destruction of the cells. Cultures of this type would be advantageous to morphological studies of virus-cell relationships.

Four primary cell lines were developed: two of bovine origin, one of swine origin and one of canine origin. All have low susceptibility to FMDV except the one of canine origin which appears to be refractory. Two of the lines were developed after suppression of the highly susceptible cells in the original cultures by viral action (in connection with ADP a8-30). None of the cultures have been carried long enough to determine if they have the indefinite growth potential of permanent cell lines. The cultures are being

observed for degree of susceptibility, conditions related to spontaneous cure of infection, and susceptibility to reinfection after cure.

(ADP a8-31)

O. Investigations of Rinderpest in Cattle

Rinderpest virus, strain, Kabete O, was attenuated in tissue cultures. An avirulent virus population was segregated using the terminal dilution technique. The virus has been studied in laboratory tests and has shown promise as a vaccine in cattle and sheep. It has not shown properties to revert to full virulence in either species. The thermal and hydrogen-ion properties of rinderpest virus have been determined using cell culture techniques.

A sample of the rinderpest vaccine developed at PIADL has been made available to a Food and Agriculture Organization Laboratory of the United Nations located in Cairo, Egypt. The vaccine is being studied in that country for its usefulness in protecting animals against exposure to rinderpest.

(ADP a8-23)

P. Studies on Foot-and-Mouth Disease Virus (PL 480 Project)

Research is being conducted on FMDV at the Instituto Biologica, Sao Paulo, Brazil. At this Institute the investigators are serially passaging certain types of FMDV in a variety of types of tissue cultures. Following passage, the viruses are examined to determine the immunizing properties and according to the results recently received from that Institute, there is evidence that at least some of the viruses are becoming somewhat attenuated for cattle. The workers at this Institute have shown that tissue cultures are exceptionally valuable in diagnosis of samples submitted from the field. Using tissue cultures as an indicator medium and saliva from affected animals as a test sample, positive identification of FMDV has resulted in a large percentage of the samples so examined. The workers at this Institute have also developed a cell line from the kidneys of swine which is useful in primary isolation of virus from samples submitted from the field. The cell line which has been developed by workers at this Institute multiplies rapidly, may be easily subcultured, and is sensitive to all of the types of FMDV against which it has been tested. It may well be useful for production of virus for commercial fabrication of FMD vaccine. (S3-ADP-2)

Q. Studies on Various Indigenous Types of Foot-and-Mouth Disease Virus, and the Production of a Vaccine for the Control of FMD in Turkey (PL480 Proj)

This investigation is in progress under a PL 480 Grant to The Ministry of Agriculture, Laboratories of Foot-and-Mouth Disease and Tissue Culture, Etlik, Turkey. The work is still in the preliminary stage, since the grant was made during the reporting period. (A22-ADP-8)

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PARASITES AND PARASITIC DISEASES OF CATTLE Animal Disease and Parasite Research Division, ARS

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of \$400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA AND COOPERATIVE PROGRAM

The <u>Department</u> has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the following designated locations.

The <u>Federal</u> scientific effort devoted to research in this area totals 21.5 professional man-years. This effort is divided among subheadings as follows:

Ecological Factors Influencing Gastro-Intestinal Nematodes of Cattle 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effect of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Acquisition and Effects of Roundworm Parasites of Cattle as Influenced by Diet 1.5 at the Animal Disease and Parasite Research Division, Beltsville Parasitological Laboratory, Beltsville, Maryland.

Cultural Characteristics and Artificial Propagation of Protozoan Parasites
1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Host-Parasite Relationship of Coccidial Parasites of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Ecology and Immunology of the Cattle Lungworm, <u>Dictyocaulus viviparus</u> 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, Including Anthelmintic Treatment 2.0 at the University of California, Davis, under a cooperative agreement with the ARS-USDA.

<u>Investigations of Trichomonad Parasites</u> 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan, Utah.

Host-Parasite Relationship of Intestinal Worms, Cooperia spp. in Cattle 2.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Etiology and Immune Response of Cattle to Winter Coccidiosis 1.0 at the Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Montana Agricultural Experiment Station, Bozeman.

Anaplasmosis of Cattle 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and through a memorandum of understanding and other agreements in cooperation with the State Experiment Stations in California, Illinois, Louisiana, Nevada, and State Veterinarian of Tennessee, the USDA Entomology Research Station, Kerrville, Texas, and the Delta Branch Experiment Station, Stoneville, Mississippi.

Interrelationships of Diet and Parasitic Infection in the Production of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Histochemistry of Gastro-Intestinal Nematodes of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Parasites of Cattle with emphasis on Stephanofilarial Species 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, University Park, New Mexico.

Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (Cattle, Sheep, and Alpacas)(PL-480 Peru)

Investigations on Anaplasmosis, Piroplasmosis and Babesiallosis of Cattle are under way through a PL 480 Grant at the School of Veterinary, Montevideo, Uruguay (PL 480 Uruguay)

PROGRAM OF STATE EXPERIMENT STATIONS

Twelve Western States and the Department are cooperating in regional research on internal parasitological problems of cattle (W-35). Informal coordination is maintained with States in the southern region also working on this subject. New and improved methods for diagnosing nematode parasitic diseases are being developed and relationships between types of pasture forages and degree of parasitism are being established. Biological and chemical controls are under evaluation. The effects which promising anthelmintics have upon weight gains and feed efficiency of parasitized cattle are being measured.

Basic studies are seeking to establish how nematodes damage the host animal, interfere with nutrition and bring about disease. Studies of biochemical systems involved in parasite metabolism and the effect of anthelmintics on these systems are providing key information necessary in developing improved therapeutic measures.

Other studies are aimed at reducing exposure to cattle parasites through the development of systems for managing grazing and feeding procedures. Factors which favor over-winter survival of infective parasite larvae are being determined and micro-climatic conditions conducive to larval infectivity are being established. Studies at several locations are in progress on coccidiosis of cattle to determine the conditions favoring outbreaks of this disease. Factors affecting immunity to this parasite are being determined. Basic information is being sought at a number of States on the nature of Anaplasma in order to elucidate the life cycle of this parasite and provide a means for its control. Preventive immunization is under study and methods of eradication are being explored.

The total State scientific effort devoted to this research is 8.9 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Etiological Factors Influencing Gastro-Intestinal Nematodes of Cattle.

- l. Investigations made at Experiment, Georgia, under the auspices of the Regional Animal Disease Laboratory at Auburn, Alabama, showed a reduction in the number of infected larvae of various cattle and sheep nematodes proportional to the increase in number of viable spores of Bacillus thuringiensis var. thuringiensis Berliner (Rohm and Hass Co., and Stauffer Chemical Co.,) incorporated in feces-worm egg cultures. Some of the same materials fed to calves and sheep proved to be toxic, without any appreciable reduction in the number of larvae obtained from fecal cultures from these hosts.

 (ADP bl-6(Rev.)
- 2. Studies have shown that a grain ration (corn) appears to have an inhibitory action on the development in the feces of the larvae of the five species of cattle nematodes (Trichostrongylus axei, T. colubriformis, Oesophagostomum radiatum, Cooperia oncophora, and C. pectinata.) Apparently, silage does not have any effect on larval development. (ADP bl-6(Rev.)
- 3. Sporangia of a mold, <u>Pilobolus</u> spp. was observed to disseminate infective larvae of nematode parasites of cattle present on the vesicle at the time the sporangia were discharged. This may constitute another way for the dissemination of nematode larvae from the fecal pad in the field to the forage to be consumed by ruminants. This report confirms earlier observations made in England. (Alabama and Georgia) (ADP bl-6(Rev.)
- B. Acquisition and Effects of Roundworm Parasites of Cattle as Influenced by Diet.

At the Beltsville Parasitological Laboratory (BPL), Beltsville, Maryland, four experiments, using a total of 37 calves, were performed to determine whether feeding a normal level or a subsistence level of combinations of grain and hay for 7 weeks preinoculation of larvae would effect their resistance to infection of gastro-intestinal worms. The calves weighed from 171 to 331 lbs. at the time of inoculation. Lots 1 and 2 received 248,000 and 116,000 larvae, respectively, of the medium stomach worm. Lots 3 and 4 received a total mixture of 485,000 and 523,000 larvae of the medium stomach worm and 6 other species of gastro-intestinal parasites, respectively. Calves were necropsied 6 to 7 weeks post-inoculation.

The parasitized calves on the low feeding level were outgained by their respective controls by 12.2 lbs./100 lbs. of TDN consumed (total digestible nutrients) (Avg. of all 4 experiments). The parasitized calves on the higher feeding level were outgained by their controls by 9.2 lbs./100 lbs. of TDN consumed. The parasitized calves on the higher feeding level outgained those on the lower feeding level by 11.8 lbs./100 lbs. TDN consumed and the uninfected control calves on the higher feeding level outgained those on the lower feeding level by 11.5 lbs./100 lbs. TDN consumed.

Analysis of these results showed that moderate infections with gastrointestinal nematode parasites reduced the efficiency of feed utilization by calves more than did mild infections, but feeding level did not appear to affect the susceptibility of the calves to infection with these parasites under the conditions of the experiments. (Maryland) (ADP bl-19 R)

C. <u>Cultural Characteristics and Artificial Propagation of Protozoan</u> Parasites.

Experiments at the Beltsville Parasitological Laboratory on the symbiotic relationship between the protozoan parasite, <u>Histomonas meleagridis</u>, and the enteric (intestinal) bacterial flora of its natural hosts, chickens and turkeys, and from several small mammals, showed bacteria from the turkey were more beneficial to the parasite than were those from the chicken; those from small mammals were without benefit. This finding may provide at least a partial explanation of why this parasite is more damaging to the turkey than to the chicken.

The probiotic activity for <u>Histomonas</u> of turkey cecal bacteria was not destroyed by heating at a temperature of 56°C (132.8°F) for 30 minutes or at lower temperatures for as long as 60 minutes. At temperatures of 65°C (149°F) or higher the activity of the bacteria was destroyed within about 5 minutes. (Maryland) (ADP b1-22)

D. Host-Parasite Relationships of Coccidial Parasites of Cattle.

The ADP Regional Animal Disease Laboratory, Auburn, Alabama, reported that for the first time observations of a preliminary nature were made on the endogenous cycles of <u>Fimeria cylindrica</u> and <u>E. canadensis</u> in calves. Schizonts, merozoites, microgametocytes and macrogametocytes of <u>E. cylindrica</u> were found in fresh smears and sections of the intestines at 8 days postinoculation. In <u>E. canadensis</u> at 15 days postinoculation, fresh smears revealed microgametocytes and macrogametocytes in the small intestine 6 and 12 feet anterior to the cecum. (Alabama) (ADP bl-23(Rev.))

E. Ecology and Immunology of the Cattle Lungworm, Dictyocaulus viviparus.

Work conducted at the Beltsville Parasitological Laboratory showed that oral vaccination of calves with infective larvae of the equine lungworm for protection against infection with the cattle lungworm was without adverse clinical effect. The procedure showed promise of efficacy. (ADP bl-24)

F. Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, Including Anthelmintic Treatment.

The School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, reported research on anthelmintic treatment with the following results:

I. ANTHELMINTIC STUDIES

- A. Trial 1. Activity of Bayer S-940 and Bayer S-6658 against nematodes in sheep.
 - 1. Bayer S-940 removed 95% and 98% of nematodes from lambs at dosages of 50 mg./kg. and 100 mg./kg., respectively.
 - 2. Bayer S-6658 removed 92% and 98% of nematodes from lambs at dosages of 200 mg./kg. and 400 mg./kg., respectively.
- B. Trial 11. Activity of Bayer 9017 and Bayer 9018 against nematodes in sheep.
 - 1. Bayer 9017 removed 65% and 90% of nematodes from lambs at 15 mg./kg., and 30 mg./kg., respectively.
 - 2. Bayer 9018 removed 17% and 38% of nematodes from lambs at 20 mg./kg. and 40 mg./kg., respectively.

II. PHYSIOLOGICAL STUDIES

- A. Iron kinetic studies in Hereford steers during the early acute phase showed no significant alterations from the normal. This would suggest that only in the later stages of the disease are alterations to be found.
- B. The biological half life of total body water in cattle suffering from acute gastrointestinal parasitism was found to be about twice that of normal animals.
- C. The serum albumin/serum globulin ratio was found to change from 0.49 in severely parasitized cattle to 1.00 twenty-five days after therapy. (California) (ADP b1-25)

G. Investigations on Trichomonad Parasites.

Research workers at the ADP Regional Animal Disease Laboratory, Logan, Utah, reported that a graded series of freeze-dried organisms was inoculated intravenously into two rabbits. Both rabbits showed anaphylactoid shock after each inoculation. One rabbit died after the third inoculation, and as a result, the other rabbit was given only four inoculations. The rabbit that died had a serum agglutination titer of 160 at time of death; the other rabbit produced an excellent serum titer of 2560. Serum from this rabbit, however, with the homologous antigen produced no precipitin lines on gel diffusion plates. Serum from the rabbit that died produced one precipitin line. Due to the high agglutination titer produced in the one rabbit, it is felt that further trials are warranted using freeze-dried T. foetus organisms intravenously.

Five experiments were run using different strains of Trichomonad foetus. A series of six inoculations of live washed Trichomonads, followed by a single large inoculation one month later, produced antisera with agglutination titers up to 2560 and 10240 in the first two experiments. The second inoculation did not increase the agglutination titer but did result in stronger precipitin reactions in gel diffusion plates. However, only two and three precipitin lines were formed. The antisera were freeze-dried and rehydrated to one-fourth their original volume. Gel diffusion reactions were considerably improved, producing 4 to 6 precipitin lines consistently. This concentrated serum also responded normally to electrophoresis and can be used for microimmuno-electrophoresis.

At the Utah Agricultural Experiment Station, Logan, research was continued under a cooperative agreement with the USDA on trichomonads and related flagellates of the bovine digestive tract. In the examination of animals, infection was found as follows: a) in the cecum of 1, and in the rumen of 1 of 45 cattle; b) in the cecum of 16 of 21 calves; c) in the cecum of 7 and in the rumen of 8 of 17 sheep; d) in the cecum of 14 of 14 pigs. Twelve pigs had <u>Trichomonas suis</u>; 7 had <u>T. buttrey</u>; and 4 had <u>Trichomitus rotunda</u>.

A pentatrichomonad was found to have the highest incidence of any flagellate in the bovine cecum and rumen. It was easily cultivated in several different media. Two strains from the rumen had similar growth curves, but in 2 clones from the cecum and 2 strains of Pentatrichomonas hominis of human and canine origin, the growth curves were distinctly different. An organism with 4 flagella arising in pairs was found in the cecum and feces of cattle, and described as Monocercomonoides bovis n. sp. (Utah) (ADP bl-26)

H. Host-Parasite Relationship of Intestinal Worms, Cooperia species, in Cattle.

Reported research from the ADP Regional Laboratory, Auburn, Alabama, showed that calves and lambs inoculated with infective larvae of either of the intestinal nematodes, <u>Cooperia oncophora</u> or <u>C. pectinata</u>, developed immunity to challenge inoculation with either the homologous or heterologous species. One animal that failed to develop immunity to the homologous species also lacked immunity to the heterologous species.

Calves inoculated with 300,000 <u>Cooperia pectinata</u> infective larvae in a single dose were killed by their infections. Two that were moribund 6 weeks after inoculation had lost 20 and 21 pounds. Calves inoculated with 10 successive daily doses of 30,000 larvae each gained an average of 7.3 pounds, while non-inoculated controls averaged a 36.7 pound gain. Calves given 42 successive daily doses of 7,140 larvae gained an average of 31.5 pounds, while controls averaged 42 pounds in one test, and 77 and 78.3 pounds, respectively, in another similar test. The results are additional evidence of the pathogenicity of <u>C. pectinata</u> and indicate that clinical

parasitism develops in susceptible calves as the result of acquisition of infective larvae in large numbers over short periods of time. (Auburn, Ala.)

(ADP b1-27)

I. Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle.

The Beltsville Parasitological Laboratory research workers reported that the larvae of the gastrointestinal worms of cattle can develop at a temperature of 35°F to the second stage when they have been preconditioned for two weeks at 45°F. This is a much lower temperature than is usually thought to be conducive to the development of the gastrointestinal nematodes of cattle.

The viability of eggs of the beef tapeworm of man was reduced by 80 and 90% by exposure to 50,000 r and 100,000 r of x-irradiation, respectively. Only 0.29% of the cysts (Cysticercus bovis), which cause condemnation and retention of carcasses for bovine cysticercosis under meat inspection regulations, developed from a large dose of eggs exposed to 200,000 r of x-irradiation. (ADP b1-28)

J. Etiology and Immune Response of Cattle to Winter Coccidiosis.

Research was continued at the Montana Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, under a cooperative agreement with the USDA. Reported observations on 10 disease outbreaks in cattle indicated that Eimeria zurnii was the predominant organism in 5 cases, E. bovis in three, and E. canadensis and E. brasiliensis each in one case. Confirmatory diagnosis of clinical coccidiosis was made in only 4 instances in which E. zurnii occurred alone. Oocyst counts on mucosal scrapings from the lower colon of the above animals varied from 19,500 to 3,612,000 oocysts/gram.

Supernatant from a saline emulsion of colonic contents of a Hereford calf that died after showing symptoms typical of convulsive coccidiosis, was highly toxic to mice when injected intraperitoneally. This supernatant was not neutralized by <u>Clostridium perfringens</u> anti-sera of types A, C, or D, or by <u>Cl. septicum</u> antiserum. This colonic supernatant remained lethal to mice, after being stored for 6 days, when injected intraperitoneally in O.1 ml. doses. (Montana) (ADP b1-29)

Studies were continued on Winter Coccidiosis at the ADP Regional Laboratory at Logan, Utah. Three experiments were conducted involving prolonged daily oral inoculation of Holstein-Friesian calves with sporulated oocysts of Eimeria bovis and E. zurnii. In one experiment calves were inoculated 50 days with 500 or 15,000 E. bovis oocysts by adding an aqueous suspension containing oocysts to the evening feeding of milk. Each calf was given a single challenge inoculation of 500,000 oocysts after recovery from the initial prolonged inoculations.

In a second experiment, similar in objectives, calves were fed 100 or 15,000 occysts daily in the evening feeding of milk, or 1000 occysts in the evening feeding of grain. A third group of calves served as controls. All calves were given a challenge inoculation of 500,000 occysts.

The results of the first 2 experiments showed that calves ingesting the least oocysts developed less severe symptoms of coccidiosis than did those ingesting the larger number. The length of time calves were susceptible to repeated inoculations was about the same in all groups and the degree of immunity was similar, although calves undergoing infections wherein large numbers of oocysts were discharged and clinical signs were severe seemed to exhibit a somewhat stronger immunity. These results confirm those reported from one experiment in last year's report.

Ten calves were inoculated with sporulated oocysts previously exposed to radiation of 10,000 r, 50,000 r, 100,000 r, or 200,000 r in a cobalt-60 source. Calves receiving oocysts irradiated at 10,000 r developed coccidiosis similar to control calves receiving non-irradiated oocysts. Those receiving oocysts exposed to 50,000 r exhibited mild coccidiosis. Those receiving oocysts exposed to 100,000 r or 200,000 r developed no evidence of coccidiosis and were completely susceptible to challenge with non-irradiated oocysts. It appeared that the sporulated oocysts exposed to 100,000 r and 200,000 r were killed by the radiation and were unable to elicit an immune response in the gut. The amount of radiation required to kill sporulated oocysts appears to be between 50,000 r and 100,000 r, probably about 75,000 r. (Utah) (ADP b1-29)

K. Anaplasmosis of Cattle

At the Beltsville Parasitology Laboratory, research workers reported the following findings: Serum samples from cattle in the incubative, acute, and carrier stages of bovine anaplasmosis were tested by the agar gel precipitin technique and the complement-fixation reaction. The agar gel technique proved to be unreliable as a supplementary diagnostic test.

A free soluble antigen of <u>Anaplasma marginale</u>, exo-antigen, was found to be produced and released into the peripheral blood of cattle with acute anaplasmosis. The significance or immunogenic potential of this material has not been determined.

Calves given an immunizing inoculation of sonicated hemolysate of anaplasmainfected RBC (red blood cell) in a mineral oil adjuvant and then challenged were only partially protected. The hemolysate conferred partial protection against the severe form of the disease but did not prevent the animals from becoming carriers. Filtration and high speed centrifugation with a sucrose gradient were employed in the examination of the complement-fixation antigen to determine whether sub-microscopic particles of <u>Anaplasma</u> occur in this antigen. Sub-microscopic units of <u>A. marginale</u> were not found.

Ten adult-to-larva and two series of adult-to-adult hereditary transmission experiments with the Rocky Mountain wood tick, <u>Dermacentor andersoni</u> Stiles, failed to transmit anaplasmosis. All test calves were found to be susceptible when challenged with anaplasma-infected blood.

Exposure of adult ticks to hibernating environments of 4°C, relative humidity of 40-50%, and of 25°C, relative humidity of 80%, had no observable effect on their transmission potential. However, ticks exposed to the low temperature fed faster and produced 50 - 70% more eggs after hibernation than did ticks held at 25°C. Nine of 49 <u>D</u>. <u>andersoni</u> males, subjected to hibernation for 263 days, survived. Of these, 5 attached and fed on a susceptible calf which failed to contract anaplasmosis. The ticks had originally fed on a calf with acute anaplasmosis 358 days before they were placed on the test (susceptible) calf.

Colonization of \underline{D} . occidentalis has been successful and the colony is now in the F_3 generation. (Maryland)

The ARS anaplasmosis research herd at Kerrville, Texas, is composed at the present time of 40 mature cows, 8 two-year-old heifers, and 27 calves nearing weaning. The last of the reactor cattle were sold last year (1963) and the herd has continued through this fiscal year as an anaplasmosis-free herd. The total number of cattle will be reduced to approximately 35 to conform to available pasture. (Texas) (ADP b1-30)

L. <u>Interrelationship of Diet and Parasitic Infection in the Production</u> of Cattle.

Research workers at the ADP Regional Laboratory, Auburn, Alabama, reported the effect of parasitosis on the basal metabolic rate of rabbits infected with either 5,000, 10,000, 15,000, 20,000, or 25,000 infective Obeliscoides cuniculi larvae, indicated that the parasitic infections established did not cause a marked difference between the basal metabolism of the infected and control animals.

Experiments on the biology and host-parasite relationship of Longistrate noviberiae and Trichostrongylus affinis in domestic rabbits indicate these parasites are well adapted for experimental use. The short prepatent period, direct life cycle, high percentage of adult worm recovery makes these ideal parasites for experimental work with diets in rabbits. (Auburn, Alabama) (ADP b1-31)

M. Histochemistry of Gastro-Intestinal Nematodes of Cattle.

The report on work conducted at the ADP Regional Animal Disease Laboratory, Auburn, Alabama, showed that 8 to 10 days after infection of cattle with the nodular worm, <u>Oesophagostomum radiatum</u>, there is a decrease in collagen around the lesions formed by the worm in the walls of the ilium. Concurrently with this decrease in collagen there is a real or apparent increase in a substance, probably glycoprotein, containing protein and carbohydrate moieties, between the lesions and the lumen of the intestine. Such histochemical changes were not observed around the sites of infection by the medium stomach worm, <u>Ostertagia ostertagi</u>, 8 days after the host calves were infected. At the above stages of these diseases, alterations in the distribution of glycogen and acidic mucopolysaccharides of the tissue were not observed.

Glycogen and acid mucopolysaccharides, as well as collagen, have been found in Obeliscoides cuniculi, a nematode of the domestic rabbit. Glycogen was principally found in the intestinal wall and in the body wall muscles. Acid mucopolysaccharides lined the digestive tract and were present in the muscles of the body wall. Collagen was seen in the cuticle, the hypodermis, and in various membranes, as well as in the gonads. (Alabama) (ADP b1-32)

N. Parasites of Cattle with Emphasis on Stephanofilarial Species.

Investigations made at the ADP Regional Animal Laboratory at University Park, New Mexico, were reported as follows: Stephanofilaria stilesi is a small filarioid nematode causing an ulcerative dermatitis along the ventral mid-line of cattle, and uses the horn fly, Haematobia irritans, as a biological vector. Horn flies were experimentally infected with the larval stages of \underline{S} . stilesi by exposing laboratory-reared flies to the lesion on infected cattle. The infective stage is reached after about 18 days of development in the fly. The biological cycle of S. stilesi was completed by exposing young calves to infected horn flies. Two calves developed lesions typical of stephanofilariasis within two weeks after their initial exposure. One calf was examined post-mortem after one month and found to be infected with immature S. stilesi. From eight to thirty-two per cent of the horn flies collected from infected cattle on rangeland, irrigated pasture, and in drylot were found to be infected with the larvae of S. stilesi. (New Mexico) (ADP bl-33)

O. Under a PL 480 Grant to the School of Veterinary Medicine, University of San Marcos, Lima, Peru, research is in progress on Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (Cattle-Sheep-Alpacas). Most of the work reported has been of the nature of a preliminary survey of multiple areas or districts to determine the kinds of parasites therein that infect animals.

P. Under a PL 480 Grant to the School of Veterinary, Montevideo, Uruguay, research is in progress on Anaplasmosis, Piroplasmosis, and Babesiellosis of Cattle. Crushing and macerating of tick larvae appeared necessary for development and evolution of the larvae, and their transformation into nymphos in vitro. A temperature of 37°C was found to be the most favorable for cell survival and development.

The development of tissular components was improved by the addition of glutamine to the media. Chicken plasma was also found to improve the media.

The pathogenicity of whole blood infected with <u>Babesia</u> <u>bigemina</u> was modified by irradiation with gamma rays at dosages above 30,000 r.

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EQUIPMENT AND BUILDINGS USED IN PRODUCING BEEF CATTLE Agricultural Engineering Research Division, ARS

Modern methods of producing livestock call for increasing use of engineering principles. More knowledge is needed about the effects of environment on animals and what structures and equipment are best suited to provide the most favorable environment. Better methods of applying pesticides to animals are needed and methods of eliminating pests without using chemicals on animals would be preferred. New and additional methods are needed for using electrical and other energy to replace costly human labor in many operations devoted to feeding and care of animals. Manure disposal, especially with confinement type operations near a metropolitan area, is becoming an increasing problem. The continuing threat of nuclear warfare demands consideration of types of building that could provide protection from fallout for livestock and their feeds and provide facilities for operation during periods of emergency. To meet present day market demand for lean-type meat, producers need objective nondestructive methods and instruments for estimating the amount of lean meat in live animals.

USDA AND COOPERATIVE PROGRAM

This is a continuing program involving engineers and architects conducting basic laboratory investigations, application of laboratory results to a production basis, and development of typical plans for livestock structures. The work is in cooperation with the Animal Husbandry, Animal Disease and Parasite, and Entomology Research Divisions of ARS, USDA. Plan development work is cooperative with all the State Colleges through Regional Committees and with the Federal Extension Service, as part of the Cooperative Farm Building Plan Exchange. The professional man-years shown in parentheses at the end of each of the following sections may include work applicable to other species of animals.

- A. Beef Cattle Engineering. Beef cattle structures and equipment for hot, dry climates is conducted in cooperation with the California Agricultural Experiment Station at the Imperial Valley Field Station, El Centro. Related studies for a warm humid climate are in cooperation with the Missouri Agricultural Experiment Station at Columbia, and with AH, ARS. (This work was inactive this year.) Typical plans for beef structures are developed at Beltsville, Maryland. (0.6 PMY)
- B. Livestock Shades and Shelters. Shades for sheltering livestock are being studied at Tifton, Georgia, in cooperation with the Georgia Station. (0.1 PMY)
- C. Reducing Pesticide Residues in Animal Products. Reduction of pesticide residues in animal products, with beef cattle receiving major attention, is studied at Kerrville, Texas, in cooperation with ENT and ADP, ARS, and the Texas Agricultural Experiment Station. (1.0 PMY)

- D. Water Supply and Wastes Disposal for the farmstead are studied at College Park, Maryland, in cooperation with the Maryland Agricultural Experiment Station. Liaison is maintained with the Public Health Service, the Water Systems Council, the American Society of Agricultural Engineers, and other organizations concerned with rural sanitation. (2.2 PMY)
- E. <u>Fallout Protection</u> work for the farmstead is conducted at Beltsville, Maryland, and selected field locations. Liaison is maintained with the Office of Civil Defense, Department of Defense, and other appropriate agencies. (1.4 PMY)
- F. Equipment and Control for Automatic Feeding of livestock and poultry is under development in Washington and Illinois State Experiment Station. Work on performance characteristics of upright-silo unloaders is in cooperation with the Minnesota State Experiment Station. (2.9 PMY)
- G. <u>Instrumentation</u>. At Beltsville a program is underway to develop and provide accurate, practical and sometimes complex instrumentation for specific program needs. (1.7 PMY)

PROGRAM OF STATE EXPERIMENT STATIONS

There is an extensive program of both basic and applied research underway at the State Agricultural Experiment Stations in an effort to provide the answers to the continuing series of questions being raised by livestock producers. Demands are being made for more information on the effects of environment on the physical well being of all classes of livestock, and the way such optimum environments can be economically achieved; on new approaches to meet the growing labor shortage; on methods to adapt existing structures and equipment for greater economy of production; and on structures and related equipment for improved efficiency of feeding and materials handling operations.

Studies are being made of the effect of environment on the health, growth, production and fertility of dairy cattle, beef cattle, poultry and swine. Equipment and systems for efficiently transporting feedstuff into and out of storages and automatically mixing and feeding complete rations are being developed.

Exploring methods for improved care and housing of farm animals with greater economy and labor efficiency are also in progress as well as investigation of ways to modify existing structures and equipment to meet present-day economic conditions.

A widespread research effort is in progress which is attempting to investigate all of the factors involved in the complicated problems concerned with disposal of farm waste materials, particularly concentrated manures resulting from confinement-type livestock operations. The problem is most acute and the public is demanding a fast solution to this unsanitary and potentially dangerous health hazard.

Studies are conducted to obtain information on uses of electrical energy and explore new uses and test equipment. Many of the projects are concerned with the varied problems of chore labor mechanization and an expansion of the use of electricity for ventilating, heating, lighting and cooling under the various production enterprises of today's farming operations. Development and testing of prototype specialized equipment for product collection, processing, packaging, and transport, as well as storage, loading and unloading devices, are a part of the overall program of investigations.

Approximately 45 professional man-years covering work on all animal species and poultry are devoted to these problems. Much of the research is conducted cooperatively with the Department.

PROGRESS - USDA AND COOPERATIVE PROGRAMS

A. Beef cattle engineering

1. Hot, arid climates. At the Imperial Valley Field Station, El Centro, California, in cooperation with the California Agricultural Experiment Station, three pens of beef cattle were used to study the effects of floor slope on weight gain, activity, and manure removal. In one pen the floor was level, in the second at a slope of 4 3/4 percent and in the third at 7 percent. Average daily gains during each of 6 test periods were:

Period	I (7%)	Slope II (level)	III (4 3/4%)
days			
28 29 28 27 28 28	0.83 2.39 1.65 2.26 2.73 1.31	1.80 1.75 1.49 0.83 2.69	1.18 2.81 1.59 1.81 2.87 1.06
Average	1.86	1.59	1.90

Manure moved readily down both the sloping floors because the pens were completely shaded and the manure did not dry out. Actually, it was planned that the animals would work the manure downhill as they walked to the upper side to the feed trough. This probably would occur if the pens were not so shaded and the manure had a chance to partially dry out. During some periods of the next test, these pens will be unshaded.

Activity studies showed the following percentages of time lying, standing, and eating:

	Pen	Lying	Standing	Eating
		percent	percent	percent
8/22-23	I	33	57	10
	II	46	43	11
	III	52	35	13
9/3-4	I	51	38	11
	II	27	65	8
	III	53	36	11
10/13-14	I	55	36	9
	II	28	66	6
	III	46.5	47	6.5

Animals on the level floor did not lie down as much because the floor was much dirtier. Pen I cattle had the smallest gain the first 28-day period, and activity studies showed them to be standing a high percentage of time. The general idea seems to work fine and these exploratory tests, along with the associated activity studies, indicate possibilities for improvement and also for application. The labor of manure removal always seems to be high.

An air-conditioned "Reference House" has been designed for the Imperial Valley Field Station and will be constructed sometime during the next year. It will be pre-fabricated, metal, and approximately 35 by 65 feet with pens for 12 animals in individual stalls.

- 2. Hot, humid, climate. Inactive during reporting period.
- 3. Plan development. At Beltsville, Maryland, working drawings were prepared for four plans covering the layout and construction details pertinent to modern range corrals for beef cattle based on field studies of successful operations. Also a design was completed for a bunker type shelter with protection features for fallout. A pressure-treated wood frame supports an earth cover overhead and on three sides. Baled hay stacked at one end provides shielding and feed for approximately two weeks. (The man-years for this plan are reported under Fallout Protection, 10 F).

All these plans are included in the Cooperative Farm Building Plan Exchange.

B. Livestock shades and shelters

At Tifton, Georgia, in cooperation with the Georgia Agricultural Experiment Station, a study was continued in an effort to define the best height for cattle shades in a hot, humid climate such as the Southeastern United States. Three shades, 12 by 24 feet, were erected—at heights of 6, 9, and 12 feet, each covered with galvanized metal. Black globe and shielded air temperatures were recorded on various days at the animal level.

Directional radiometer traverses were made under the 6-foot and 12-foot shades. The average black globe temperature was reduced 19.6° F. under the 6-foot shade and 17.5° F. under the 12-foot shade at an air temperature of 89.3° F. and an unshaded black globe temperature of 114.2° F. The average radiant heat load was 172.8 Btu/hr/sq.ft. under the 6-foot shade and 179.9 Btu/hr/sq.ft under the 12-foot shade. It was concluded that the radiant heat load on animals in the Southeast is greater under high shades than under low ones, and there is no thermal comfort advantage for shades over 6 feet high. Also, it seems reasonable to expect that the amount, frequency, and type of clouds would dictate the optimum height of shades in a particular area.

C. Reducing pesticide residues in animal products

Development and testing of automatic sprayers for cattle were continued because the experimental devices offer methods of providing practical and efficient control of flies with fewer residues of insecticides in meat and milk than other spraying equipment. Work is cooperative with projects ENT-h2-l, "Development of Insecticides, Repellents, and Other Materials and Methods for the Control of Horn Flies, Stable Flies, and Face Flies" and ENT-m11-2, "Development of Methods of Analysis for Insect Control Chemicals". Laboratory tests with the experimental sprayers indicated that residues of the insecticides, D.D.T., Ronnel, and G.C. 4072, left in milk by the low volume treatments, were significantly less than those of more conventional high-volume treatments. Daily automatic sprayer treatments of 80 to 120 ml. (.02 to .03 gal.) left residues not exceeding four parts per billion in milk. Concentrations that were approximately as effective as more conventional 1/2 or 1 gallon treatments left only 0.7 to 2.5 percent as much residue.

Field tests conducted during the summer of 1963 indicated that the low-volume application of Ciodrin (Shell 4294) by the experimental sprayers provided satisfactory control of horn flies on cattle. The data verified the conclusion, reached during the previous year, that the experimental sprayers controlled horn flies as well as more conventional equipment with significantly less insecticide. Operation of the sprayers under field conditions provided detailed information useful in establishing guidelines for the design of sprayers or other equipment for the self-treatment of cattle. A number of modifications with simplified and improved design were developed.

A simplified procedure for measuring the quantity of Co-Ral on the hair of treated livestock was developed in cooperation with project ENT-mll-2. The simplified procedure was used for determining the distribution of spray on a cow treated by an experimental automatic sprayer. Preliminary analysis of the results indicated that the method was quite suitable for evaluating boom and nozzle systems.

D. Farmstead manure disposal. Laboratory and field studies are continuing in Maryland, in cooperation with the Maryland Agricultural Experiment Station, on the characteristics of animal manures that affect their handling and disposal and on developing design criteria for disposal lagoons. Laboratory work has shown that a potable, sanitary "water" can be produced from manure lagoon effluent by chemical disinfection. The process should be within the means of many farmers. Observation of soil sealing and sludge buildup rates in an operating hog manure lagoon in Maryland substantiated previous laboratory findings of 39 days sealing time in a "Manor" soil and 1 mm. per month sludge buildup. Preliminary investigation of the effects of irradiation of lagoon liquids with radioisotopes indicated that it is apparently possible to sterilize the liquids with low-level radiation and that algae cells are rendered non-reproductive for varying periods.

The major portion of a manuscript for a publication on farm animal manure disposal was prepared.

E. Fallout protection

At Beltsville, Maryland, work continued on development of plans and guide materials for fallout protection structures to be included in the Cooperative Farm Building Plan Exchange. Eight typical plans were developed for protective structures for farm families, animals, and crops.

A bunker type shelter for beef cattle was developed which features the economy of an earth cover overhead and on three sides. Baled hay stacked in one end provides shielding and feed for approximately two weeks. A pressure-treated wood structure supports the earth cover.

F. Beef and Dairy Cattle Feeding Equipment

In Illinois, work cooperative with the University of Illinois Department of Agricultural Engineering has progressed on the automatic silo unloader control for dairy and beef cattle feeding systems. A 3-position (floating control) and 2-position control was tested at hoist speeds of 5-30 inches per minute. The 3-position control worked well at all hoist speeds. The 2-position control worked best at low hoist speed and with a current differential of .1 ampere or less. With a current differential of 1.0 ampere the discharge was erratic at all hoist speeds. The severity of variation increased as hoist speed increased.

In Minnesota the performance of electric motors for the operation of silo unloaders is being determined in cooperation with the University of Minnesota Agricultural Engineering Department.

An intensive electric motor testing program was continued during the past year. The requests for information in this area seem to be growing. The capacitor motors which the unloader industry discontinued six years ago were completely tested to give a base from which to predict required performances. Only one new repulsion-start induction-run motor was introduced during the past year while several capacitor motors were introduced. At the moment only two capacitor motors appear to have been redesigned to meet the requirements of this application. Several others are only reintroductions of general-capacitor motors of the type which proved to be unsatisfactory several years ago.

Upon completion of the laboratory tests, motors with questionable performance characteristics are placed in daily operation in a silo for six months.

Several motor manufacturers are now offering their new designs for testing under this project.

In a cooperative project with the Washington State University Agricultural Engineering Department the development of an automatic trench silo unloader has progressed. The unloader has a power requirement of 9-1/2 horsepower, operates in silos with irregular side walls, has the capacity of a vertical silo unloader and can be controlled manually or automatically. Under automatic control it cuts a 12-inch by 1-inch slice from the face of the silage at the rate of 100 pounds per minute. It can operate in all types of silage.

G. Instrumentation Research

Ultrasonic reflectance measurements were continued on hogs, cattle, and sheep for correlation with yields of meat cuts. Longissimus dorsi thickness of cattle and sheep were statistically combined with liveweight and compared with area and weight measurements from cattle and sheep. The multiple correlation of ultrasonic measures and liveweight were: with cattle Longissimus dorsi area, 0.35; with cattle weight yield of round, rump, and loin, 0.95; with sheep weight of trimmed or untrimmed leg, 0.95. Yield predictions, based on liveweight, were improved by adding the ultrasonic measures. A recently available bio-medical pulse-echo instrument was trial used and compared with existing unit. Some advantages were observed in respect to convenience; however, the operation principle is basically the same as presently used equipment.

PUBLICATIONS - USDA AND COOPERATIVE PROGRAMS

Beef Cattle Engineering

- Agricultural Engineering Research Division. 1963. Hay storage and feeding shed. (Exchange Plan No. 5935). Mixcellaneous Publication No. 938. July. (Also listed under A, Dairy Cattle Engineering)
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- Bond, T. E. 1964. Response of animals to environment. Proceedings of Association of Southern Agricultural Workers, p. 41 (abstract). February.

Livestock Shades and Shelters

- Hahn, G. L., Bond, T. E., and Kelly, C. F. 1963. Relation of cooling indices to electrical energy use of air-conditioned farm structures. ASAE Transactions 6(3):241-243, 248.
- Hahn, G. L., Bond, T. E., and Kelly, C. F. 1963. Walls influence interior radiant environment of livestock shelters for shade. California Agriculture 17(9):10-11. September.

Reducing Pesticide Residues in Animal Products

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Wastes Disposal

Eby, Harry J. 1963. Manure disposal lagoons. ARS 42-75. June

Fallout Protection

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- Petersen, D. R. 1964. Cutter unit for horizontal silo unloader. Transactions of the ASAE. Mar. 7(1):6-7.
- Puckett, H. B., and Daum, D. R. 1964. Control system for automatic silo unloading. Agricultural Engineering Journal. Jan. 45(1):26-27, and 35.

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II. NUTRITION AND CONSUMER USE RESEARCH

NUTRITION AND CONSUMER USE RESEARCH

Consumer and Food Economics Research Division, ARS
Human Nutrition Research Division, ARS

The assortment and characteristics of foods available to consumers Problem. are constantly changing with the adoption of new production, processing, and marketing practices. Constantly changing also, as nutrition science advances, is our understanding of the nutritional needs of man and the manner in which these needs can best be met by food. To help meet the Department's responsibility to advise consumers on the quantity and variety of foods that will assure maximum benefit and satisfaction research must continue on the nutritional requirements of persons of all age groups, and on the nutrient and other values of foods and on how to conserve or enhance these values in household preparation and processing. Periodic surveys of the kinds and amounts of foods consumed by different population groups and individuals also are essential for evaluation of the nutritional adequacy of diets and to give the guidance needed for effective programs of nutrition education. Information from such surveys provides assistance needed in market analyses for different commodities and in the development and evaluation of agricultural policies relating to food production, distribution, and consumer use.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program of research concerned with (1) nutritive and other consumer values of raw and processed foods as measured by chemical or physical means and by biologic response; (2) effects of household practices upon the nutritive values and inherent qualities of foods, and the development of principles and improved procedures for household food preparation, care, and preservation; (3) surveys of kinds, amounts, and costs of foods consumed by different population groups and the nutritional appraisal of diets and food supplies; and (4) development of guidance materials for nutrition programs.

The research is carried out by two divisions of the Agricultural Research Service -- the Human Nutrition and the Consumer and Food Economics Research Divisions. Most of the work is done at Beltsville and Hyattsville, Maryland; some is done under cooperative or contract arrangements with State Experiment Stations, universities, medical schools, and industry. The total Federal scientific effort devoted to research in these areas total 63.3 man-years. It is estimated that approximately 5.9 man-years is concerned with studies related to beef products.

Human metabolic studies and the related exploratory and confirmatory studies with experimental animals and microorganisms concerned with defining human requirements for nutrients and foods are not reported on a commodity basis, though some of the work is applicable to this report. This basic nutrition research represents a total Federal effort of 26.7 professional man-years and is described in detail in the report of the Human Nutrition Research Division.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Nutrient Value of Food

Food composition and nutritive value are most frequently related to indigenous agricultural products. Specific and locally grown raw products are being extensively evaluated for essential nutrients, especially in Hawaii and Puerto Rico. Much work is related to changes induced by growing practices, processing and storage.

The form of fats and lipids in food stuffs and the changes involved in processing and holding are receiving special attention as the role of different types of fat in human nutrition unfolds. Protein content and structure continue as active research areas.

Certain raw products are being evaluated for their significant vitamin contribution to nutrition. The effect of production and processing practices on vitamin content continues as an area of interest. Additionally, research has been directed toward the study of vitamins in food stuffs as affected by inhibitory and stimulatory factors.

The total program in this area includes 36 projects in 23 States and is comprised of 23.4 professional man-years.

Properties Related to Quality and Consumer Use of Food

In the area of food preparation, products are related to quality by some measure. Special measures characterize certain classes of products; ie., vitamin assays, enzymatic activity, water binding capacity, and changes in structural tissues. Combinations of these are involved in the quality evaluation work reported.

The major research in product development is on the production, processing and storage of beef, pork, lamb, poultry and eggs. Variables which affect the initial product, include feeding regimens, age and breed, are under study. Conditions of processing relate to freezing temperature, storage temperature and time, shelf life, and the effect of light.

Other research includes the quality of meat tenderness as influenced by chronological age, post-mortem aging and in relation to connective tissue. Genetic factors which may be operative in establishing carcass characteristics is being investigated in sheep.

Food preparation research focusing on products for home use include: Heat penetration of meats and baked products and the chemical changes involved; microwave preparation of meats, fruits and vegetables, including the chemical alterations involved; and flavor characterization in frozen and stored products by means of vapor component identification.

Many of these same factors are under study in institutional preparation where the quantities involved impose special conditions. Heat penetration and internal temperature as related to quality, yield and culinary quality is an area of intensive study.

This portion of the program includes 52 projects in 21 States and is comprised of approximately 50.1 professional man-years. This is a partial report of the State Experiment Station programs in food science and includes work undertaken by home economics departments. For research on food and fiber utilization see reports of the Utilization Research and Development Divisions.

Food Consumption and Diet Appraisal

The State program in food consumption and dietary appraisal extends the work of the Department to other segments of the population or to geographic areas not separately identified in the nationwide studies. Currently 12 States are contributing to this program. One regional project is designed to yield information regarding food purchase and consumption patterns of families with preschool children. This group represents about one-fourth of the households in the North Central Region where the study is being made. Food habits will be evaluated in terms of the children's dietary needs. This research will provide information useful to both consumer and market interests.

The State program in this area totals 22.2 professional man-years.

PROGRESS--USDA AND COOPERATIVE PROGRAMS

A. Nutrient Value of Food

1. Tables of food composition. Research for the newly revised Agriculture Handbook No. 8 "Composition of Foods...raw, processed, prepared" has been supplemented by further research during the year and adapted to the needs of special projects.

Data from several large recent studies have made it possible to develop a procedure for obtaining representative figures for calories and for the proximate and physical composition of beef. By this method data may be obtained for (1) beef carcasses or sides of the different grades, (2) retail cuts made from the carcasses with an ordinary amount of trimming, (3) retail cuts completely trimmed of all separable fatty tissue, and (4) cooked meat prepared from the retail cuts. The method as developed provides data having appropriate relationships between the physical composition (percentages of lean, fatty tissue, and bone) and the proximate composition (percentage of water, protein, fat, ash). The data on beef published in Handbook 8 were obtained by this procedure. Included in a research report are data on the physical and chemical composition of more grades and cuts of beef than it was feasible to include in the Handbook and an explanation of the procedure used in developing the values. Information presented in the report illustrates the types of research needed for meats other than beef to provide data equally satisfactory for them.

Formulas and procedures that were used in calculating the nutritive values of 250 food items commonly prepared at home are being summarized in a publication for special users, particularly therapeutic dietitians and medical research workers. A table showing average adjustments for vitamin losses during cooking has been developed and will be included in the publication.

Selected data from revised Handbook No. 8 have been made available in decks of punched cards and magnetic tape for research workers. Available in these forms are the data from Table 1, the nutritive values for 100 grams edible portion of the foods; from Table 2, nutritive values for one pound of food as purchased; from Table 3, selected fatty acids in foods. Arrangements have been made for the sale of the cards and the tape by a private data processing firm in Washington.

Tables for the Department of Defense have been prepared on the composition of 630 food items procured by the Defense Supply Agency for feeding military personnel. Values for the composition of foods developed for Handbook No. 8 and many additional values provided by the Department of Defense were used to develop the data needed for the numerous food products meeting military specifications.

2. <u>Vitamins</u>. Analyses for the vitamin B6 content and distribution in meats, including beef, and in vegetables available to and as eaten by consumers, are in progress. Analyses of cereal foods, fruits, nuts, and cheeses are nearly completed and manuscripts are in progress.

A fluorometric procedure for the determination of pyridoxine as pyridoxal cyanohydrin was developed. The reactions were quantitatively reproducible over a range in concentration of 1 millimicrogram to 1 microgram per milliliter. Procedures for chemical assay for pyridoxal and pyridoxamine previously had been developed in this laboratory. Present studies are to

adapt chemical procedures to analyze food extracts for the three forms of vitamin B6. The procedures are expected to provide a more constantly reliable method for measuring this vitamin. Values from the chemical procedures are being compared with values obtained by microbiological determinations for vitamin B6 in foods.

Development of coordinated procedures for B-vitamin analyses continued with emphasis on a rapid, stable chemical method for nicotinic acid.

3. <u>Lipids</u>. Studies under contract with the University of Tennessee, on the changes in the fatty acid composition of fat in meats when cooked, are estimated to be about half completed. These studies are on ground beef and pork patties using fatty acid composition of glyceride and phospholipid fractions as determined by gas-liquid chromatography, and infra-red spectra for indications of changes due to heating.

B. Properties Related to Quality and Consumer Use of Beef

- 1. Endpoint temperatures and quality of broiled beefsteaks. Internal temperature to which rib or eye-of-round steaks are heated during broiling had more influence on scores for tenderness, juiciness, and flavor than factors of marbling of the carcass or fat content of the muscle. As steaks were broiled to higher internal temperatures, they were usually scored less tender, less juicy, and less flavorful. Eye-of-round steaks broiled to an internal temperature of 140° F. were considered by the panel to be medium done rather than rare; those cooked to 160° or 180° F. were considered well done. Rib steaks, on the other hand, were considered rare, medium, and well done when cooked to 140°, 160°, and 180° F., respectively. Research is being initiated to determine the effect of rate and extent of heating by broiling on color changes in beef during cooking.
- 2. Freezer preservation of meat in the home. A publication on "Freezing Meat and Fish in the Home", was prepared in cooperation with the U. S. Department of Interior. It presents the latest recommendations on freezing techniques, storage time, thawing, and cooking. The bulletin points out that for high-quality frozen food it is necessary to have home freezing equipment that freezes food quickly at 0° F. or lower and maintains these temperatures for storage of frozen products. Too high or constantly changing storage temperatures cause even frozen foods that are properly packaged to lose quality and food value. Illustrations show how to cut and bone beef, pork, and lamb and how to wrap these products for the freezer.
- 3. Use of agricultural chemicals. Rib roasts from beef animals sprayed with Ruelene had more off-flavor than rib cuts from animals sprayed with water. Flavor of ground round, liver, and kidney from Ruelene-treated animals was similar to flavor of corresponding cuts from control animals

and was not adversely affected by the treatments. Two Ruelene treatments were evaluated: (1) beef animals sprayed five times with 0.5 percent Ruelene (0-4-tert-butyl-2-chlorophenyl 0-methyl methylphosphoramidate) and (2) sprayed once with 0.75 percent Ruelene. Results are to be published in the Journal of Economic Entomology.

4. Food distribution program. Revision of the publication "Quantity Recipes for Type A School Lunches" (PA 631), was completed in cooperation with the Agricultural Marketing Service and the Fish and Wildlife Service, U. S. Department of Interior. This recipe card file provides 324 quantity recipes or variations and other information needed in preparing Type A lunches in schools participating in the National School Lunch Program. Recommendations on preparing, storing, and handling a wide variety of cereal, dairy, fruit, vegetable, meat, and poultry products were updated to take into account recent research findings and technology. New recipes were laboratory tested and taste panel evaluated, and all formulas and serving yields were recalculated in line with the 1964 revision of PA-270, Food Buying Guide for Type A School Lunches.

C. Food Consumption and Diet Appraisal

1. Planning for proposed nationwide survey, households and individuals. A nationwide survey of household food consumption and of the food intake of individuals is scheduled for 1965. Plans have been developed for a survey that would provide at least 6,000 household schedules and 10,000 individual schedules in the spring of the year with smaller household samples in each of the three succeeding seasons. The information on the week's food use to be obtained from each household is similar to that obtained in 1955, except that information on home baking practices will not be requested and information requested on home food production, home canning and home freezing will be reduced to allow interview time for questions on the food intake of individuals in the households.

In preparation for the proposed first nationwide survey of the food intake of individuals, data obtained by recall on the 1-day intake of food from nearly 550 individuals of all ages in Washington, D. C. during June and July 1963, have been studied in relation to two controversial issues that concern collection of data. The survey findings indicate that for this group: (1) the nonresponse rate on food intakes from individuals is not influenced by taking a schedule on household food consumption first in comparison to taking none, nor is it influenced by taking a schedule on food intakes from half in comparison to all individuals in the family; and (2) homemakers report the amounts of food eaten by family members in terms of their individual servings far more often than as proportions of household amounts. Tabulations of the Washington data also are useful as a pretest for tabulation of the nationwide survey.

- 2. Effects of food distribution programs on diets of needy families. A survey of the food consumption of more than 800 households that were not participating in the food stamp program in St. Louis was made in May and June 1964 to determine the relation between usual family food expenditures and payments required for food coupons. Homemakers were asked also why their families did not participate in the program. Results of the analysis will guide the Department in revamping the St. Louis stamp program to make it more acceptable to eligible families and yet keep it within the limits of the program. Because of interest in the nutritional quality of food consumed by low-income families, an assessment may be made later of the dietary levels of these families. This is the sixth in a series of USDA food program surveys made in cooperation with the Marketing Research Division, ERS to assist the AMS to administer the food stamp and direct distribution programs.
- 3. Food consumption of the rural population in Spain (PL 480 research). A survey of the food consumption of the rural population in Spain has been initiated by the Spanish Ministry of Commerce under the cooperative sponsorship of the Economic Research Service and the Agriculture Research Service, using PL 480 funds. The study will provide information needed in appraising potential markets in Spain for U. S. farm products and should yield information useful to U. S. authorities on efficient ways of improving nutrition in low-income areas. The Spanish Ministry of Commerce expects to obtain much useful information on which to base a program for improving the diets of rural families, especially through better distribution of food. Information on food consumption, income levels, and related socio-economic characteristics has been obtained from about 1,200 rural families in 6 major regions of Spain. In summarizing the results, emphasis is being placed on (1) determining the nutritional shortages among these rural families at different income levels in the different regions, and (2) computing income elasticities for different foods as well as total food consumption.
- 4. Nutritive value of national food supply. The nutritive content of the per capita food supply is calculated each year from estimates of quantities of foods consumed (retail weight basis) as developed by the Economic Research Service. This series, which begins with the year 1909, is being completely revised to incorporate newest estimates of per capita consumption, revised food composition data from Agriculture Handbook No. 8, and new information on the nutrients added to foods by enrichment and fortification.

A survey conducted by the Bureau of the Census for the Consumer and Food Economics Research Division has provided information for the years 1957-61, on quantities of enrichment ingredients supplied to processors to fortify flour and cereal products. Through this program about one-third more thiamine, one-fifth more iron and niacin and one-tenth more riboflavin are added to the Nation's diet than would be available if foods were not enriched.

For the first time, the enrichment survey was extended to include information on the quantities of ascorbic acid and vitamins A and D added to foods, thus furnishing a base line for future surveys. Currently the amount of ascorbic acid added to foods would be enough to increase the level in the per capita food supply by 5 percent. The contribution from synthetic vitamin A is 7 percent of which 6 percent is added through margarine. Vitamin D is not at present included in nutrient estimates.

- 5. Household practices in home freezer management. Recording forms and questionnaires for obtaining data on management practices of urban and rural home freezer owners were pretested and necessary revisions were made in preparation for data collection among households in Fort Wayne and a nearby rural area. Information will be obtained in two seasons on the kinds, amounts, sources, prices, and turnover rates of frozen foods stored in the home. Such data will provide information needed to develop guidance materials for improved management of home freezers.
- 6. Development of food budgets and other basic data for food and nutrition programs. Interpretation of nutrition research findings and their application to practical problems has continued as part of an ongoing program to assist nutritionists, teachers, health workers, and other leaders concerned with applied nutrition programs or nutrition policies. Information developed under this program is provided to many groups both within and outside the Department working on practical food programs, on questions relating to nutritional requirements, food consumption, nutritional importance of specified foods, and on nutrition education. Increased emphasis has been given this year to opportunities for disseminating information to the public through TV and radio, the press, conferences, workshops, and the Department's Food and Home Fair.

Food budgets at different cost levels for individuals and families are priced quarterly for publication in <u>Family Economics Review</u> as a continuing service to welfare workers, extension agents, and others needing this information. For example, in June 1964, the cost of one week's food for a family of four including 2 school-aged children, was estimated to be \$24.40, \$32.80, and \$37.40, respectively, for the low-cost, moderate-cost, and liberal plans.

The food budgets published in Home Economics Research Report 20, "Family Food Plans and Food Costs," have been reexamined in the light of revisions in food composition data (Handbook 8, revised) and in recommended dietary allowances of the National Research Council. Some modification in food quantities was needed for certain individuals. This has necessitated revision of food plans and their presentation in technical and popular publications, including Agriculture Handbook 16, "Planning Food for Institutions," now being readied for publication. The "Food Purchasing Guide for Group Feeding," formerly a part of Agriculture Handbook 16, is in the final stages of editing for publication as a separate handbook.

All other existing guidance materials for nutrition programs were reviewed in light of the changes in recommended dietary allowances and in food composition data. Some publications have been revised; others will be updated for the next reprinting.

Nutrition Program News, a bimonthly periodical prepared for members of State nutrition committees and other community nutrition workers provides one channel for disseminating pertinent information about Federal programs and for reporting nutrition activities in the States. Issues this year included such diverse subjects as a report of the World Food Congress held in Washington, June 1963, "Labels on food products—the protection they give," and "Nutritional fitness for teenagers." Assistance to workers in nutrition programs has been provided also through consultation and program participation by staff nutritionists.

PUBLICATIONS--USDA AND COOPERATIVE RESEARCH

Nutrient Value of Food

- Watt, B. K., and Merrill, A. L. April 1964. Composition of Foods...raw, processed, prepared. Agriculture Handbook No. 8. Revised December 1963. 190 pp.
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- Consumer and Food Economics Research Division. 1963. Conserving the Nutritive Values in Foods. Home and Garden Bull. 90. 16 pp.
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III. MARKETING AND ECONOMIC RESEARCH

MARKET QUALITY OF BEEF Market Quality Research Division, ARS

Problem.

Meat is a very perishable commodity which varies greatly in quality characteristics such as tenderness, juiciness, flavor and fat content. To the consumer the visual characteristics of meat quality are primarily color and fat-to-lean ratio. However, properties such as tenderness, juiciness and flavor cannot be judged so simply. The meat grader attempts to evaluate these quality factors by relating quality to evidences of maturity, texture of the lean, and degree of marbling. To insure more uniform grades and standardized products, better objective tests for measuring the quality attributes of tenderness, juiciness and flavor in meat are needed. Also needed are more effective methods for maintaining optimum quality by minimizing such deleterious effect as shrinkage and bloom and by enhancing the shelf-life of meat as it moves through market channels.

USDA PROGRAM

This work is being conducted at Beltsville, Maryland, with the cooperation of the Animal Husbandry Research Division, ARS, the Livestock Division, AMS, and in part by research contract with the University of Missouri and by cooperation with the University of Illinois. Research programs concerned with the development of new techniques for measuring meat tenderness and for evaluating the composition of livestock, carcasses and meat cuts are underway. The application of the ultrasonic technique to estimate the thickness of backfat and muscling in live hogs, cattle, and sheep is one example of this type of research. Another area of interest is concerned with the use of improved sanitary practices in the merchandising of meat to extend shelf-life and to develop objective methods for the evaluation of quality and shelf-life of prepackaged fresh meats. Studies are also underway to standardize lighting conditions in work areas where meat grading is done.

To augment in-house research at Beltsville a new meat laboratory has been established. Here instrumental techniques in conjunction with classical methods of organic and biochemistry are applied to problems concerned with the evaluation and maintenance of meat quality. Basic information gained at the molecular level concerning proteins, electrolytes, phospholipids,

triglycerides and other meat constituents will be used in attempts to establish objective methods for quality evaluation.

A grant with Robert College, Istanbul, Turkey, provides for the development of an odor-measuring instrument for use in inspection and grading of foods. Its duration is for 5 years, 1961-1966, and involves P.L. 480 funds with a \$29,361 equivalent in Turkish liras.

A grant with the Research Center of the Meat Industry, Helsinki, Finland, provides for a study on the effects of carbon dioxide or nitrogen on refrigerated meat. Its duration is 4 years, 1963-1967, and involves P.L.480 funds with a \$44,453.40 equivalent in Finnmarks.

The USDA scientific effort devoted to research in this area totals four professional man-years of which one man-year is on contract and 2.5 man-years are in the area of objective measurement and evaluation of quality.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Research directed to increasing our understanding of the market quality of meat has been a continuing part of the State stations' research program. Both basic and applied research are involved.

Market quality research on meats begins with study of the influence of breeding, feeding and management treatments with cattle, sheep and swine on the carcass and meat quality characteristics. The objective is to determine the relationships of live animal and management factors to ultimate eating quality. Such live animal traits as birth weight, rate of gain, efficiency of gain by sire groups, body measurements such as depth and length of body, type, market weight and grade are related to carcass traits such as loin eye area, muscling characteristics, amount and distribution of fat, yield of wholesale cuts, chemical composition and carcass value in an effort to define animal traits which influence carcass and meat quality.

Other research involves investigation of various pre-slaughter treatments on the carcass quality, organoleptic characteristics and market value of the meat. Special attention is given to tenderness of meats and the fundamental causes of toughness or tenderness in meats. Certain post-mortem factors including aging exert profound effects on meat quality and considerable effort is devoted to attempts to gain a better understanding of their effects.

Almost all of the studies involve a certain amount of work on methods since methodology is of vital importance in the study of quality factors. Development of objective criteria for evaluation of meat quality is a continuing

goal and new and improved methods of defining the quality of meat cuts are constantly sought.

Further along the route to the consumer, concern arises as to the effects of processing and storage treatments on the quality of meat. The influence of maturity, marbling, methods of aging and processing and storage, packaging and distribution are all studied for possible effects on ultimate quality. Microbial quality, distribution of muscle proteins and lipids, morphological features, amount of connective tissue, and cooking treatment are other factors considered in attempting to establish the total quality characteristics of meat. Finally, the relationships of raw and cooked meat quality to consumer preference are determined. These are in turn related to the carcass quality and market value of the live animal.

A total of approximately 17.7 professional man-years are devoted to market quality research on meats.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Objective measurement and evaluation of quality

1. Yield of Lean Meat from Cattle of Different Conformation. The study in cooperation with the Livestock Division and the University of Illinois comparing two groups of cattle of different conformation but of the same USDA Quality Grade (Choice) has been completed. Results showed that there were no differences between the yield of lean meat from these two groups of beef carcasses when evaluated according to the system of determining yield developed by USDA. Palatability studies comparing these two groups of carcasses showed no difference in eating quality as judged by a six-member taste panel or by Warner-Bratzler shear determinations. Analysis of the beef carcass data in order to develop correlations and multiple regression equations that can provide guidelines to measurements that account for the greatest amount of variation in yield of lean meat from these wholesale cuts have also been completed.

(MQ 3-34)

2. Measurement of Tenderness. In a study being conducted cooperatively with Animal Husbandry Division the tenderness of cooked loin steaks and rib roasts of beef, representing a range in carcass grade from Utility to Choice are being measured. Subjective tenderness evaluation data are being obtained by taste-panel judgements; objective measurements by Warner-Bratzler shear determinations and measurement on puncture and shear using the Slice-Tenderness Evaluator (STE) developed by USDA. This phase has not reached the stage for reporting findings.

(MQ 3-34)

- 3. Relationship of Marbling to the Palatability of Beef. This project has been initiated to study the relationships between marbling and composition, concentration and distribution of lipid material in beef muscle. Marbling plus this type of knowledge, or this information alone, may provide a more object method for the evaluation of palatability than marbling per se. This project is in its initial stages and no findings can be reported.

 (MO 3-60)
- 4. Flavor Studies to Provide a Basis for More Objective Measurements of Meat Palatability. This project has been initiated to develop objective procedures for identifying and evaluating flavor characteristics of meat by studying the compounds and precursor systems responsible for meat flavor. Studies on beef and lamb are underway. A fraction has been isolated from unheated lamb fat that possesses characteristic lamb aroma. This crude fraction has been partially separated and procedures for the quantitative collection of these sub-fractions developed.

(MQ 3-61)

- 5. Objective Methods for Measuring Maturity. Stages of physiological maturity should be reflected in differences that can be measured at the molecular level in muscle tissue. A comparative study of the proteolytic activity of tissue, from similar muscles, from animals of different chronological age has been initiated in order to see if this measure of enzymatic activity can be correlated with maturity. New analytical procedures are being now developed in order to carry out the objectives of the research project. (MQ 3-62)
- 6. Odor-Measuring Instrument. This project covers research being undertaken in Turkey under P. L. 480 funds. The investigator came to the United States and discussed the development and research basis for his instrument at a scientific meeting held in Washington on odor measurement.

(A22-AMS-1(a))

- B. Quality maintenance in handling and packaging
- 1. Shelf-life of Prepackaged Meats. The University of Missouri has completed several storage cycles for beef and pork, under the contract, to study the factors affecting the shelf-life of prepackaged meats. Each cycle included different sanitation levels under controlled cutting room temperatures. As a result of this work a revised manual containing new recommendations for temperature, humidity, sanitation and handling procedures for fresh meats is being prepared.

(MQ 2-75)

Properties Related to Quality and Consumer Use

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- Eisen, J. N. 1964. A note on orthogonal polynomials applied to treatment levels with unequal replications. Food Science 29(1): pp. 105-108.
- 1963. Freezing meat and fish in the home. Home and Garden Bulletin No. 93, 23 pp., illus. (Animal Husbandry Research Division, Human Mutrition Research Division, and Bureau of Commercial Fisheries, Department of Interior cooperating.)
- 1964. Canned beef in family meals. Agricultural Marketing Service. Human Nutrition Research Division cooperating. AMS-530, 2 pp. (Processed).
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Food Consumption and Diet Appraisal

- Adelson, S. F., 1963. A 2-week vs. a 1-week Reference Period in Surveying Diets of Individuals. Abstract in Proceedings of Sixth International Congress on Nutrition, Edinburgh: p. 477.
- Consumer and Food Economics Research Division. 1963. "The Nutritional Review." National Food Situation, No. 106, Outlook Issue, Nov.
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- Redstrom, R. A., Davenport, E., and Murray, J. 1963. Consumer Practices in the Handling and Storing of Commercially Frozen Foods, Two Cities, Two Seasons. Home Economics Research Report 23, 27 pp.
- Consumer and Food Economics Research Division. Cost of 1 Week's Food at Home. Family Economics Review. Sept., Dec. 1963; April, July 1964.
- Consumer and Food Economics Research Division. 1964. Family Food Budgeting...for Good Meals and Good Nutrition. Home and Garden Bull. 94.
- Nutrition Program News (periodical, 5 issues): July-Aug. 1963; Sept.-Oct. 1963; Nov.-Dec. 1963; Jan.-Feb. 1964; Mar.-June 1964.

C. Quality maintenance during transportation

1. Effect of Atmospheres of Carbon Dioxide and Nitrogen on Properties of Refrigerated Meat. The first annual report (covering period April 1, 1963 - March 31, 1964) was received under this P. L. 480 research grant. During the report period only one test series with meat kept in normal atmosphere was carried out. The greater part of the report period was consumed in procuring equipment and in trial runs and in developing the chemical and bacteriological methods of analysis.

(E8-AMS-5(a))

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Objective Measurement and Evaluation of Quality

Carpenter, Z. L., R. G. Kauffman, R. W. Bray, and K. G. Weckel. 1963. Factors influencing quality in pork. B. Commercially cured bacon. Jour. of Food Science 28(5):578-583. (MQ 3-9(c))

Feinstein, Louis and Richard L. Hiner. 1963. Anesthesia and its relationship to body composition. Annals of the New York Academy of Sciences 110:1141-1145. (MQ 3-34)

Hornstein, I. and P. F. Crowe. 1964. Meat flavor - a review. Jour. of Gas Chromatography 2(4):128-132. (MQ 3-61) livestock and meat - marketing facilities, ${\cal V}$ EQUIPMENT AND METHODS

Transportation and Facilities Research Division, ARS

Problem: Many of the livestock and meat slaughter and warehouse facilities occupied today are obsolete and the work methods that can be used in such facilities are antiquated. As a consequence, labor costs are excessive and they are increasing. Many firms still are occupying facilities designed primarily for handling rail receipts and rail shipments even though the majority of these products today are moved by motortruck. This situation also adds to handling costs. Numerous firms are occupying "makeshift" facilities which were designed for other uses or for work methods and operations of a bygone era when labor costs were low. Changes in transportation systems, population growths and shifts, and advancements in technology also have brought about changes in the types of facilities -- such as livestock auction markets, commercial feedlots, and hotel supply houses. Most private firms handling livestock and meat lack the technological and engineering skills necessary to plan and develop suitable facility layouts and designs and to select the types of equipment needed. Therefore, engineering and related research is needed to provide guidelines for industry to increase efficiency including the designing of improved plant layouts which will provide proper arrangement of work areas to minimize travel distances and excessive handling and the development of work methods that will permit use of mechanized and automated equipment rather than the relatively high-cost manual methods now used in many plants.

1/ The work described here is part of an overall program aimed at improving market facilities and market operations. As agricultural commodities flow through marketing channels they converge with similar products, for example, meat, poultry, fish and dairy products are often handled by the same wholesaler and reach consumers through the meat and produce department of retail stores. Because of this situation, improvements in the overall marketing process can bring about benefits that affect several commodities simultaneously. The component costs of marketing have been rising rapidly and would have risen more if the results of this type of research had not been available. In the marketing of food commodities in 1963, at least \$30 billion (75% of the total food marketing bill) were expended on marketing operations that are directly affected by the research covered in the overall program. The overall program includes (1) terminal wholesale marketing planning, (2) preliminary and followup work in terminal market areas, and (3) production area and independent marketing facilities such as that described here. Terminal wholesale market planning was conducted in 7 major cities last year. Production area and independent market facilities planning involved 41 studies. For additional information see "A Summary of Current Program and Preliminary Report of Progress" dated September 30, 1964, by the Transportation and Facilities Research Division, ARS, USDA.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program devoted to planning marketing facilities in which application is made of engineering, economic and marketing principles. This work is concerned with structures, equipment, containers, devices, work methods, and operating methods used in marketing and transporting farm and food products from farms to consumers. The functions to which these physical elements, handling methods, and labor relate include essentially all marketing operations, especially those directly applicable to the commodities in the physical sense such as assembling, preparing for market, processing, packaging, precooling, loading, transporting, unloading, storing, warehousing, and wholesale and retail distribution. The part of the program pertaining to beef involves industrial engineers, agricultural economists, meat scientists engaged in both basic and applied research to develop new and improved methods, equipment, processes, and facilities for livestock markets, meatpackers and wholesalers. Livestock market research is carried on at Hyattsville, Maryland. Part of the work in this area is being done in cooperation with the Missouri Agricultural Experiment Station, Columbia, Missouri, and the Central Missouri Livestock Auction, Mexico, Missouri. Work on the development of a mechanical driving device and penning system for livestock markets is under a contract with the American Research and Manufacturing Corporation, Rockville, Maryland. The research on livestock slaughtering and on meatpacking and wholesaling at Stillwater, Okla., is cooperative with the Oklahoma Agricultural Experiment Station.

The Federal effort devoted to research in this area during the fiscal year 1964 totaled 5.1 professional man-years: 2.1 man-years (including 1.8 man-years of contract work) on livestock marketing, 2.3 man-years on meat facilities, and 0.7 man-year on program leadership.

PROGRESS - USDA AND COOPERATIVE PROGRAMS

A. Automation of Sales Operations on Livestock Markets

At the Central Missouri Livestock Auction, Mexico, Mo., the combination electronic load-cell and lever-system scale, and the scoreboard for flashing total weight, average weight, and price to the audience continues to perform well. The manual-key input device for transmitting sales data from the auctioneer's box to the office and the computer for processing this information could not operate at the speed of the fastest sales transaction. During the year the Toledo Scale Corporation, which provided this equipment, abrogated the Memorandum of Understanding covering this part of the work.

The contractor's report from the Toledo Scale Corporation on the physical and economic feasibility of electrically-operated gates was favorable and provided information on gate structure, latching designs, drive systems, and remote controls. A cooperative agreement was negotiated with the Missouri Agricultural Experiment Station for constructing and testing of prototype electrically-operated pen gates. Working drawings, based on data in the contractor's report, were provided the cooperator. Construction of the prototype gates was underway at the end of the report year and laboratory tests and modifications will continue for several months. Tests of the gates under actual operating conditions will be conducted on the Central Missouri Livestock Auction, Mexico, Mo.

B. Determining Behavioral Patterns of Livestock

Under a contract with the American Research and Manufacturing Corporation, Rockville, Md., research to establish behavioral patterns of cattle, hogs, and sheep under environmental conditions existing on stockyards and auction markets was conducted on a site leased from the Baltimore Union Stockyards, Baltimore, Md. The research involved determining the reaction of each species of livestock to (1) light rays of different candlepower, intensity, and bands of the spectrum, (2) sound of different pitch and intensity, (3) air blasts of different velocities and temperatures, (4) electricity applied at different voltages and by various means, (5) a moving "sweep" or "driver" of alley width equipped with selected devices, including rubber fingers, for prodding animals, and (6) selected combinations of the media listed above. The purpose of this research was to determine the feasibility of driving and penning livestock automatically.

The results of the experiments showed that a mechanical sweep with electrically charged bars was the most feasible device or stimuli for driving and penning livestock. No favorable reaction was obtained from the experiments with light rays. These included mercury vapor lamps, flashing xenon lamps, colored lights (red, blue, green, and yellow), and infrared-heat lamps. Reaction to the experiments with sound ranged from moderate to good. White noise produced the least favorable reaction, sinusoidal sound was better, and the amplified human voice was the best of the sound stimuli. Sound was eliminated from consideration as a possible driving device because of the possible effects on other livestock in the market other than those being driven and its irritation and possible painful effect to humansin the market area. Air blasts were considered a relatively good driving stimulus but were less effective than the mechanical sweep with electrically charged bars. The results of the experiments with the mechanical sweep with electrically charged bars were considered sufficiently favorable to proceed with construction and testing of a prototype driving device.

C. Developing an Automatic Driving and Penning System for Livestock Markets

A contract was negotiated with the American Research and Manufacturing Corporation, Rockville, Md., to design, construct, and test a mechanical driving and penning device for livestock markets based on the results of the research on animal behavioral patterns. At the end of the report year the contractor had submitted design drawings of the device in accordance with the requirements of the contract and was proceeding with construction of the prototype.

D. <u>Developing a Physically Integrated Livestock Marketing and Slaughtering</u> <u>Facility</u>

Due to lack of personnel, no work has been done on this project. Research in this area would draw heavily on the results of previously completed livestock marketing and slaughtering work and would require personnel who had either participated in this work or had gained from other sources a broad and comprehensive working knowledge of the engineering and technical skills needed to carry on this work. Personnel qualified to work on this project have been lost due to transfer or reassignment and it has not been possible to employ suitable replacement personnel to do the work. In view of these circumstances, the project has been discontinued until such time as qualified personnel are available to carry out the work.

E. Layouts and Work Methods for Hotel Supply Houses

At Stillwater, Okla., a draft of a report entitled "Hotel and Restaurant Meat Purveyors--Custom Service Houses--Improved Methods and Facilities" was revised to include suggestions made by industry representatives to make the report of more value to and more easily understood by the operators of hotel supply houses. At the end of the year, the report was in Branch clearance.

A draft of a second manuscript covering frozen portion control hotel supply houses was almost complete at the end of the year. This report covers receiving and storing fresh and frozen primal and boneless cuts of meat, fabricating and packaging steaks and chops, preparing ground meat and forming meat patties, freezing packaged products, casing and storing frozen products, and loading out cased products. The report compares the relative efficiency of the various work methods, and equipment types used in performing inplant operations. Use of the lowest cost methods in a plant handling an average of 75,000 pounds of meat and meat products weekly would reduce labor and equipment costs about \$15,500 or 17 percent. Most of the savings are in the labor costs and are due to the use of mechanized equipment such as conveyors, dump-buckets, patty machine feeders, and forklift trucks. An efficient layout was developed based on the lowest cost methods and equipment for this size plant.

F. Layouts and Work Methods for Cattle Slaughtering Plants

At Stillwater, Okla., a manuscript entitled "Cattle Killing Floor Systems and Layouts" was completed and published. The significant results of this study were covered last year.

PUBLICATIONS - USDA AND COOPERATIVE PROGRAMS

Layouts and Work Methods for Cattle Slaughtering Plants

Hammons, Donald R. 1964. Cattle Killing Floor Systems and Layouts.

Marketing Research Report No. 657. 50 pp.

COOPERATIVE MARKETING Farmer Cooperative Service

Problem: Farmers are expanding their use of cooperative marketing. There are constant changes in transportation, processing, and distribution technology, and in market organization and practices, and changes on the farm itself. In view of these developments, farmer cooperatives and other marketing firms require research results to perform both efficiently and effectively. Such research can assist farmers to maintain and strengthen their bargaining power, increase efficiency, and meet the quality, quantity, and service needs of today's food and fiber marketplace.

Cooperative marketing is a major way for farmers to get maximum returns from their products in the current and rapidly changing market. Farmers own and control cooperatives specifically to increase their income from crops and livestock. Gains are not automatic, however. Cooperatives must plan, develop, and actually manage the specific marketing program and services that will yield the most for their members. Marketing cooperatives must know what the market demands. They must be able to compute the probable cost of different ways of serving the market. They must understand the possibility of major economies in a well coordinated joint sales program, and understand the methods and potentials of bargaining. Management must achieve minimum costs through improved organization, good use of existing plant and personnel, and the selection and use of new equipment and methods.

USDA PROGRAM

The Department conducts a continuing long-range program of basic and applied research and technical assistance on problems of marketing farm products cooperatively. Studies are made on the organization, operation, and role of farmer cooperatives in marketing. While most of the research is done directly with cooperatives, the results are generally of benefit to other marketing firms. The work is centered in Washington, D.C. Many of the studies, however, are done in cooperation with various State experiment stations, extension services, and departments of agriculture.

Federal professional man-years devoted to research in this area totaled 23.3. Of this number, 1.0 was devoted to cooperative marketing of citrus, 2.7 to cotton, 4.5 to dairy, 1.2 to deciduous fruit, 2.2 to grain, 3.9 to livestock and wool, 1.3 to oilseeds and peanuts, 1.0 to potatoes, 3.5 to poultry, 0.1 to rice, 0.6 to tobacco, and 1.3 to vegetables.

Research also is conducted under contract with land-grant colleges, universities, cooperatives, and private research organizations. During the period of this report, contract research was performed by universities and colleges in Florida, Iowa, Louisiana, Montana, North Dakota, and West Virginia, and by one private research company.

STATE EXPERIMENT STATIONS PROGRAM

The State stations maintain a very broad research program in commodity marketing, the findings of which are valuable to cooperatives and to other marketing firms. There are at this time nine projects in eight States that deal specifically with cooperative marketing. Five projects are commodity oriented and deal with grain, tobacco, milk, livestock, and fruits and vegetables. These projects seek to find out how cooperatives are adjusting or might better adjust to changes in market structure and marketing practices. In some instances researchers are studying the success and failure of cooperatives and the organizational structure. One study of the history of major cooperative marketing associations in the State will be published as a book and will undoubtedly receive nationwide attention.

Because of the growing interest in the role of cooperatives in market structure, one State recently initiated a major project in this area. The project leader views cooperative enterprises as a structural dimension of farm markets. The objectives and operating procedures of cooperatives will be studied to see if they have a unique impact upon market conduct and performance. If so, this may have significant implications for Government policies and programs.

The total research effort on cooperative marketing in the eight States is 3.4 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Potentials in cooperative marketing

In several commodity areas an appraisal is needed of the present and potential role of cooperative marketing. Current information on cooperative operations can be related to production and marketing conditions. This research will yield suggestions about cooperative operations and services, and provide current data needed by cooperative leaders and others for planning and implementing cooperative marketing programs.

<u>Livestock</u>. Trends in consumption and the market potential for meat in the Northeast were studied on a regional basis. Several concerns were contacted in the region that buy meat for additional processing. Preliminary findings indicate that existing and proposed cooperative slaughter plants in the Corn Belt area could find profitable outlets for their members' livestock in the Northeast.

Studies under contract with Montana, North Dakota, and Oregon relating to cooperative feedlots have been completed. These studies indicate possible benefits to livestock growers and grain producers from feeding locally produced animals and crops over selling both the feeder animals and grain.

B. Improving operating methods in processing and storage

Studies were underway in several commodity fields to examine new methods, equipment, facilities, and structures for efficient and safe processing and storage of agricultural products by cooperatives.

<u>Livestock</u>. Three livestock marketing cooperatives were studied to find ways such cooperatives might increase their volume by attracting large producers, thus reducing operating costs.

C. Cost and efficiency

Research studies were undertaken to develop more efficient marketing practices and procedures through analysis of costs involved in using various kinds of facilities and methods of operation.

<u>Livestock</u>. Analysis was made of the feasibility of livestock producer cooperatives integrating their operations from production through feed-yards, marketing, processing, and distribution. Information was provided several groups about one or more phases of handling livestock.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Fox, R. L. 1964. Service--The Merit Basis for Livestock and Wool Co-ops. News for Farmer Cooperatives (Jan.).

MARKET POTENTIALS FOR NEW PRODUCTS AND USES Marketing Economics Division, ERS

Problem: Increased emphasis should be placed on new products and new uses because of their importance in expanding markets and maintaining a high rate of economic growth. Agricultural producers and processors need to take maximum advantage of the opportunities offered with respect to additional outlets for surplus supplies, increased returns, lowered costs, and improved competitive positions relative to non-agricultural products. Continuing evaluations are needed of the commercial feasibility and market potentials of new or improved agricultural products, by-products, and products from new crops in food, feed, and industrial uses; of the economic feasibility of developing new uses and establishing new crops, including appraisal of their impact on present markets; and of the economic and technical requirements of end-uses. Such evaluation will provide a sound economic base for decisions on commercial developments, as well as information to guide further utilization research by physical scientists.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving agricultural economists and personnel with dual economic and technical training engaged in research to bridge the gap between laboratory developments and commercial adoption to assist producers to realize more rapidly and more fully benefits of lowered costs, increased returns, and expanded markets that new products and new uses can afford. Research is carried on in industrial and food uses at Washington, D.C., and six field offices -- agricultural economists are located at each of the four Utilization Research and Development Divisions, New Orleans, Louisiana; Albany, California; Philadelphia, Pennsylvania; and Peoria, Illinois. Economists are also stationed at the Hawaii Agricultural Experiment Station, Honolulu, Hawaii, and at the Department of Agricultural Economics, Clemson University, Clemson, South Carolina.

Research is conducted on animal products, cotton, grain and forages, oilseeds, horticultural crops, new crops, and on impacts of technological innovations. Cooperative research is conducted with the Hawaii Agricultural Experiment Station on Kona coffee and Hawaiian fruits and vegetables, with the Pennsylvania Agricultural Experiment Station on maple products, with the Louisiana Agricultural Experiment Station on a new sweetpotato product, and with Clemson University on market potentials for modified milk. Producer groups, such as the Louisiana Sweetpotato Commission and the Michigan Apple Commission, contribute to studies of potentials of new products pertaining to their area of interest.

The Federal scientific effort devoted to research in this area totals about 19.9 man-years. Commodity-wise, 4.7 man-years are currently devoted to animal products; 3.1 to grains; 2.6 to oilseeds and sugar; 3.9 to horticultural crops; and 5.6 to other research, principally new crops and impact of technological innovations.

PROGRAM OF STATE EXPERIMENT STATIONS

Little, if any, research in economics is carried out in this area by State agricultural experiment station personnel. Much research is being conducted on the development of improved products and uses, but it is in the area of technology.

PROGRESS -- USDA AND COOPERATIVE PROGRAM

- 1. Market Possibilities for Hides and Leather. The growing threat to hides and leather use by synthetic products has led to an intensified research effort to find ways to improve the competitive position of the hides and leather industry. One avenue of investigation in cooperation with the industry is an appraisal of the economics of a modified hide trim. A field test has been made on 120 hides to determine the feasibility of cutting hides into segments and processing only the highest value portion of a hide. Preliminary analysis indicates there is a significant improvement in tannery efficiency and the grade of finished leather when bellies and trim are removed from a hide prior to tanning. Corollary investigations are also going forward to ascertain if uses of economic value may be made of the parts of the hide that are not used for leather.
- 2. Potentials of Soaps, Detergents, and Surfactants from Fats and Oils. Research on the economic potential of soaps, detergents, and surfactants made from fats and oils has been completed. Detergent makers expect to be able to use petroleum raw materials at little increase in cost in the manufacture of soft detergents to combat pollution problems. If the new soft detergents are technically competent in cleansing power and degradability, prospects are not bright for increasing volume or prices for fat materials in this use unless laboratory research develops new fat materials that will surpass competing materials in cleansing power or other functions. An item of significance at least in foreign markets is that the government committee on water pollution by detergents in Great Britain has come out in favor of a detergent composition with a 50/50 mixture of a petroleum base and a natural fat base.

3 Fats in Feeds. Brief reviews are made from time to time to check conditions and progress in markets previously researched but which are of continuing importance. A change in the source of data collected by Census on consumption of fats in feeds reveals that this market for fats is much larger than had been reported. Revised figures upped consumption of inedible tallow and grease in feeds by 76 percent in the first quarter of 1964 and indicate the use of 800 million pounds this year. A study of market potentials for fats in feeds, reported in MRR 498 (Sept. 1961), projected that fat use would reach to an annual rate of 1.4 billion pounds by 1970. Reported use in subsequent years did not show an increase over the 1961 rate even though all research evidence indicated otherwise. By collecting data from renderers instead of feed manufacturers, Census is now obtaining a more accurate coverage of tallow and grease disposition. Other fats still not reported, such as poultry oil and hydrolyzed foots, would swell total use by almost another 100 million pounds a year. The feed market now outranks the soap market as an outlet for fats and oils in both price and volume.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

- Speel, H. C., and Poats, F. J. April 1964. Economic Potential of Soaps, Detergents, and Surfactants Made from Fats and Oils. AE Report No. 53. 18 pp.
- Thompson, John W. January 1964. Hide and Leather Situation, 1964. ERS-161. 4 pp.
- Thompson, John W. July 1964. Recent Changes in Hide Marketings.

 <u>Livestock and Meat Situation</u>. 2 pp.
- Thompson, John W. May 1964. A Guide to Lower Costs and Greater Efficiency in Curing Cattle Hides. AE Report No. 54. 20 pp.

MERCHANDISING AND PROMOTION PRACTICES Marketing Economics Division, ERS

Problem: Problems of selling efficiency, consumer acceptance, orderly distribution, and coordination have grown in scope and complexity as major changes have occurred in the production, processing, and distribution of farm products. Because of the wide array of products made available to consumers, through self-service retailing, as well as other factors, merchandising, promotion, the control of product distribution and movement, and management decision-making have increased in importance as basic and essential functions in the marketing of farm products.

Because of self-service not only must a product offered in todays' supermarket be its own salesman but also it must compete directly or indirectly with thousands of other items for consumers expenditures. Substantial sums are being spent by farm groups, food processors, and retailers in merchandising and promotional efforts.

Information is needed by producer groups as well as distributors at other levels in the marketing channels to determine the effectiveness of alternative promotion, merchandising and advertising techniques, levels of promotional intensity necessary for maximum sales response and the characteristics of products lending themselves to promotional stimuli. There is a basic need for development of principles or guidelines broadly applicable to agricultural commodities and which may be used in developing and strengthening commodity promotion. More effective merchandising of farm products is also required if demand is to be influenced and greater consumer acceptance gained for farm products. Sales of individual products have become increasingly dependent on in-store merchandising that will attract consumers and influence purchases. This is particularly true for many farm commodities which are not pre-sold through intensive advertising.

Because of increased complexity of operation, firms processing and distributing farm products need information which will assist in improving management efficiency. Smaller firms and particularly those operated by producers often do not have the resources or experience necessary to develop the information or techniques necessary for more efficient operations.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing long-term program of research in merchandising, management analysis, product distribution, and promotion evaluation to provide information which can be used by producers, handlers and distributors in strengthening and expanding markets for farm products. The merchandising research program is designed to quantitatively evaluate the impact of selected selling practices and price policies on the demand for agricultural products. Specific studies have as their objectives the development

of income-expenditure elasticities and measurement of other factors influencing demand, determination of consumer and market profiles, and evaluation of alternative merchandising techniques such as packaging, display, pricing, featuring and product variation on consumer purchases.

Research relating to promotion and advertising includes studies to: determine organizational structure and procedures of commodity promotion groups for optimum control, coordination and conduct of their program; measure levels of advertising and promotional intensity required to influence sales, evaluate relative effectiveness of alternative promotional appeals, themes, and techniques, and develop principles applicable to the promotion of farm products.

Studies of product distribution, such as availability, movement of products into consumption, and profiles of markets and consumers, provide information by which sound advertising, merchandising and management decisions can be made. In addition, management type studies are conducted to provide techniques and procedures which can be used to coordinate the diverse marketing functions and improve efficiency of firms distributing farm products. Most merchandising and promotion studies are conducted in close cooperation with producer or industry groups, food wholesalers, and retailers. Industry groups giving direct financial support to research during the year include, the American Dairy Association, the Florida Citrus Commission, and Florists' Telegraph Delivery Association.

During fiscal 1964, approximately 14.5 professional man-years were directed to the area of merchandising and promotion. Of this total, 2.0 were devoted to dairy; .3 to beef; .3 to poultry; 1.1 to grains and forage; 3.5 to citrus and subtropical fruits; 1.1 to deciduous fruits and tree nuts; 2.0 to flowers, ornamentals and shade trees; and 5.2 to cross-commodities.

The research effort is centered in Washington, D. C., with professional employees stationed at State Experiment Stations in Washington and Indiana. Cooperative studies are being conducted with the following State Experiment Stations: Arizona, Indiana, Ohio, and Washington. Many studies involve data collection on a national basis while others involve case studies, and controlled experiments in selected locations.

PROGRAM OF STATE EXPERIMENT STATIONS

Much of the research at the State agricultural experiment stations in the area of merchandising and promotion is carried out in connection with specific commodities and thus reported under those headings. That reported here is problem-oriented and only incidentally commodity-oriented. Thus, to get the total effort in this area one would need to add that reported under the specific commodity sections of this report.

Consumer acceptance, preference and attitude studies represent an on-going phase of the State program. Current work deals with food and fiber items

as well as nursery products. This research is undertaken for the purpose of market test and development and, thus, is closely allied with the product development phases of the Stations program. In the year reported, this research totaled 11.4 professional man-years.

Research on consumer motivation and decision-making is underway at 15 State stations. These studies are concerned with type and amount of influence resulting from food promotion and consumer information programs, with factors affecting food purchase decisions, and with consumer behavior in the market place. Limited work is also underway on improved consumer grades for agricultural products. This phase of the market development research totals 17.7 professional man-years.

Research Example -- Children's Role in Influencing Food Purchases, Miss. Project 1238.

While children's influence upon family food practices is readily accepted, there is little knowledge about the nature and extent of their role. The Mississippi Agricultural Experiment Station reports that nine- and ten-year-olds are like their elders in that they know somewhat more about food needs than they put into practice. The usual reason for not eating needed foods was that they were not provided by the family. The children studied learned about food from many sources, especially those involving social activity. Market promotion schemes (labels, coupons, and premiums) seemed to have little effect upon them. They reported that requests of parents to buy a food were usually granted, and that all types of food were requested--meat, Vegetables, sweets, and soft drinks. This study concluded that the role of the mother in influencing children's food patterns should not be underestimated.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

(1) Economics of Pricing, Merchandising, and Labor Utilization in Retailing Meat and Meat Products. Analysis of labor utilization in the retail meat department shows wide variation in costs of processing whole cuts into retail cuts. These findings point up the potentials of more effective planning and coordination in the overall operation of the meat department and stress the necessity of analyzing individual commodity costs in appraising pricing efficiency. Additional data are being collected to determine the impact of special features on store, department, and commodity sales and to develop prediction equations. These data along with that previously collected will be used to appraise the pricing efficiency of the retail marketing system for meats and develop techniques that will reduce marketing costs and increase sales of meat products.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

MARKET STRUCTURE, PRACTICES, MARGINS, COSTS, AND EFFICIENCY Marketing Economics Division, ERS

Problem: The purpose of this research is to find solutions for economic problems in marketing dairy, poultry, and meat animals and their products. More specifically, it is to find answers to the needs of farmers, marketing agencies, and the public for economic knowledge about these commodities--needs for economic knowledge that is relevant to marketing decisions and to the shaping of public policy and programs. This project includes studies of margins, costs and efficiency; of the structures of the systems for marketing individual products; and of the methods and practices followed by farmers, marketing firms, and related public agencies. It provides accurate information about the form and working of the marketing system as a basis for initiating desirable changes and for keeping all parts of the system abreast of technological and economic progress.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program of economic research to assist farmers and marketing agencies to adapt to changes in the environment in which they operate. Work in this area is conducted at Washington, D. C. and in cooperation with State agricultural experiment stations at Durham, N. H., Athens, Ga., St. Paul, Minn., Ames, Iowa, Fort Collins, Colo., Still-water, Okla., and College Station, Texas. The Federal scientific effort devoted to economic research in this area totals 33.3 professional man-years, distributed as follows: dairy 10.0, swine 0.4, beef 0.5, livestock (crosscommodity) 10.7, and poultry and eggs 11.7. By functional areas, it is distributed as follows: structures, practices and competition 15.0, product quality 3.1, information, outlook and rural development 0.7, and margins, costs and efficiency 14.5.

PROGRAM OF STATE EXPERIMENT STATIONS

All the State experiment stations are conducting economics research dealing with the marketing of animals and animal products.

Livestock marketing research deals with the economic problems involved in the marketing of beef, swine, sheep and wool. The major part of the work is in the areas of structures, practices and competition; merchandising and promotion; product quality; margins, costs and efficiency and transportation. Four regional livestock marketing projects are underway. NCM-25 investigating needed adjustments in production relative to prospective demand and is determining the effect of production, consumption and transportation costs upon market structure. WM-39 is designed to show the nature and extent of

direct marketing, and costs and returns from different methods of marketing. WM-48 is concerned with the market organization of the livestock industry in the West and is studying Western and Central markets for feeder and fed cattle and the volume-cost relationships for meat distribution. SM-23 is investigating meat and livestock movements and the role of transportation cost regarding the location of livestock production and processing facilities.

The State effort devoted to livestock marketing research amounts to 32.02 professional man years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Information, Outlook and Rural Development

1. Effects of Shrinkage on Pricing Cattle. Test lots of cattle were followed from Colorado feedlots through marketing channels to packing plants in Denver and Greely, Colorado, and were weighed at the feedlot, twice at the market (on arrival and when sold) and at the packing plant.

Shrinkage averaged 1.61 percent of initial weight from feedlot to yards or to packer direct, 1.60 percent during stay in the stockyards. Time in transit had largest relation to shrinkage loss, 0.13 percent additional loss for each 10 minutes additional transit time. Shrinkage averaged higher for heifers than for steers. Dressing percent averaged higher for both heifers and steers sold direct to packers, compared to those sold through stockyards.

The 4 percent shrink deducted from sale weight on direct sales was 1.25 percent more than shrink on sales through the stockyards.

B. Structure, Practices, and Competition

- 1. Competitive Position of Texas-Oklahoma. Potential fed beef markets for Texas and Oklahoma are mostly in the South and Southeast. Texas and Oklahoma are at a locational disadvantage with respect to fed beef markets in the Northeast and California. The major competitors of Texas and Oklahoma for fed beef markets in the South are Kansas, Missouri, and Colorado.
- 2. Outlets for Western Livestock. Studies of feedlot, country market, and terminal market prices for slaughter cattle, indicate no one market outlet consistantly furnishing highest prices at all times. Under most conditions, Choice grade steers and heifers were priced higher at country than at terminal markets, with slaughter cows the opposite; and feedlot prices in direct purchases were highest of the three methods.

Larger ranchers were found better suited than small ones to direct marketing, having enough feeder cattle to sort into uniform lots and therefore to gain reduced out-of-pocket costs on cattle shrink in hauling, at the market, and carcass shrink at the packing plant, and on procurement and selling cost under alternative methods of sale.

Analysis of rail and truck rates for hauling cattle in the West showed significant differences between rail and truck by length of haul, average weight per haul, and total weight. Rail rates tend to be lower than truck rates for hauls longer than 200 miles although truck hauls are as much as twice as fast as most rail haulers. Transportation cost was not, however, the prime factor in choice of markets by producers.

3. <u>Simulation of Pricing and Marketing Livestock</u>. Alternative pricing and trading strategies were incorporated into a computer model of the livestock-meat economy, and the prices and outputs under these alternative structures were simulated over both the historical and projection periods. Three forms of wholesale-to-retail margin strategies were simulated--a constant percentage markup, a semi-variable markup containing both a fixed component and a percentage markup component, and a fixed markup which varied with the price index. The variable margin strategy is preferable to the fixed margin inasmuch as the fixed margin restricts output and shows more extreme price cycles. The variable markup (percentage markup) produced a lower average wholesale-to-retail margin than either the semi-variable or fixed margin strategy.

Both a fixed (1958-62 average) and a 4 percent limitation on net foreign trade in beef reduced net imports from 40 to 60 percent. Trade limitation increased the amplitude of the price cycle, but raised average wholesale beef prices approximately one dollar per hundredweight over the 11-year projection period. However, average per capita beef consumption was about one pound lower during the 1964-75 period. This raised the average Choice grade steer price 75 cents over the projection period. Pork prices and per capita consumption of pork were not affected to any significant extent.

A "product utilization control" strategy which maintained a target per capita consumption rate with a two-price system for beef and pork essentially eliminated the price and output cycle. Wholesale beef and pork prices averaged one dollar higher than the historical structure projection in the 1964-75 simulation, and per capita consumption was also slightly higher. However, the Nation would be a major exporter of pork under such a program.

<u>Under the assumption</u> that the 30 day producer holding action lowered the 6 month average price substantially due to the increased marketings of the following 60 days, the long-run effects over the following 9 years showed total production to be about the same with slightly lower prices. The amplitude of the beef and pork price cycles was increased. The buildup in the cattle cycle, as measured by January 1 inventories, was held down for several years. The major result of this simulation is to show the nature of the long-run effects associated with a major short-term market change.

C. Margins, Costs and Efficiency

1. Marketing Costs and Margins. Changes in price spreads for beef consisted of two parts: Short-run fluctuation and long-term trend. The short-term changes were associated closely with the lag between adjustments in farm and retail prices. The long-term trend was persistently upward at a greater rate than spreads for other meats have increased, and at a greater rate than present indicators of retailer costs or the consumer price index. A possible explanation of this trend is a change in retailer pricing policies which shifts overhead from other commodities to beef.

Meatpacker costs for slaughtering hogs and distributing fresh pork averaged about 3 1/2 cents for wholesale pound output for two quarters in 1962-63. Total costs did not differ appreciably with changing volume, in two quarters, for this sample. About two-fifths each of total labor cost was for killing hogs and cutting carcasses; the remainder for over-filling and shipping room expense. Costs for curing and smoking hams, bacon and picnics amounted to an additional 5 to 15 cents per pound of cured and smoked product.

Meatpacker costs for slaughtering and distributing fresh beef per wholesale pound averaged about 3.1 cents for plant costs and an additional 1.1 cent for shipping to distribution centers and local delivery.

2. Economies of Scale in Meatpacking. The analysis indicated that average cost of slaughtering cattle decreases slightly as plant size increases from 20 to 60 head per hour and increases slightly for plants designed to operate, with a single shift, at line speeds of 75, 90, and 120 head per hour. The average cost estimates for operation at rated line speed were \$6.96 per head for the 20 head per hour plant, \$6.86 per head for the 40 per hour plant, \$6.50 per head for the 60 per hour plant, \$6.65 per head for the 75 per hour plant, \$6.72 per head for the 90 per hour plant, and \$6.89 per head for the 120 per hour plant.

Short-run average costs increased for each size of plant as output increased from 90 to 115 percent of rated line speed. Over the range of plant sizes studied, the average cost decreased an average of \$.47 per head as plant output increased from 90 to 115 percent of rated line speed.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

- Agnew, D. B. December 1963. Meat packers' costs: Recent interest, methods of analysis and implications. Jour. Farm Econ. 5 pp.
- Motes, W. C., Bullock, J. B., and Hacklander, D. C. May 1964. Price spreads for beef, ERS-182. 7 pp.

COMMODITY SITUATION AND OUTLOOK ANALYSIS Economic and Statistical Analysis Division, ERS

PROBLEM

Because of the instability of the prices he receives and rapidly changing conditions of agricultural production, the farmer stands in special need of frequent accurate appraisals of his economic prospects if he is to plan and carry out his production and marketing activities in an efficient and profitable way. The typical farmer cannot afford to collect and analyze all the statistical and economic information necessary for making sound production and marketing decisions. It is a goal of the Department to provide the farmer with economic facts and interpretations comparable to those available to business and industry. This is accomplished through a continuous flow of current outlook information, the development of longer range projections of the economic prospects for agricultural commodities, and analyses of the economic implications of existing and proposed programs affecting farm commodities.

USDA AND COOPERATIVE PROGRAM

The program includes the regular publication of 12 commodity outlook reports; holding of the Annual Outlook Conference in Washington in mid-November; participation of commodity specialists at regional and State outlook meetings and at meetings of farm organizations and agricultural industry groups; preparation and publication of special articles bearing on both the short-run and long-run outlook for farm commodities; issuance of comprehensive statistical bulletins containing the principal economic series pertaining to the various commodities; long-range projections of supply of and demand for the major agricultural commodities; and continuing analysis of the impact of existing and proposed alternative farm programs as they affect output, utilization, and prices of these commodities.

Except for a Regional Field Office for Livestock, in Denver, Colorado, all the USDA situation and outlook work is carried on in Washington. The regional livestock project is a cooperative effort including the Economic and Statistical Analysis Division, the Federal Extension Service, and State Extension Services in the Western and certain Great Plains States.

The total USDA commodity situation and outlook program currently involves 21.5 professional man-years.

(a) <u>Livestock and Meat</u>. This work involves 2.5 professional man-years in Washington and 2.0 professional man-years in Denver, Colorado. The outlook and situation program provides a continuing appraisal of the current and prospective economic situation of livestock and meats. These appraisals, developments of interest to the industry, and results of special studies

are published of times a year in regular issues of the Livestock and Meat Situation, in special additional issues as warranted, quarterly in the Demand and Price Situation and the National Food Situation, and monthly in the Western Livestock Round-Up, which is supplemented by special releases and materials circulated to Extension Marketing Specialists in the cooperating Western and Great Plains States. A comprehensive analysis of the livestock situation is presented at the Annual Outlook Conference. Outlook appraisals are presented at regional and State outlook meetings, at meetings of farm organizations, and to various agricultural industry groups. Special analyses are prepared on the probable effect of proposed feed grain programs on the price, supply and consumption of livestock and livestock products. Basic statistical series are maintained, improved and published for general use in statistical and economic analysis. A Statistical Handbook, Livestock and Meat Statistics is published annually.

(b) Fats and Oils. This work involves 2.0 professional man-years in Washington. The outlook and situation program provides a continuing appraisal of the current and prospective economic situation of fats, oils, and oilseeds. These appraisals, developments of interest to the industry, and results of special studies are published 5 times a year in the Fats and Oils Situation, quarterly in the Demand and Price Situation and the National Food Situation, and occasionally in monthly issues of the Farm Index and the Agricultural Situation. A comprehensive analysis of the fats and oils situation is presented at the Annual Outlook Conference, and more limited appraisals are given at meetings with industry groups. Special analyses are prepared on the probable effect of proposed programs on the acreage, price, supply, and demand for oilseed crops and for fats and oils and their products. Basic statistical series are developed, maintained, improved and published for general use in statistical and economic analysis. A Statistical Handbook, Oilseeds, Fats and Oils, and Their Products, 1909-63, is being revised and updated for publication in the fall of 1965.

PROGRAM OF STATE EXPERIMENT STATIONS

For the most part the States depend upon the U.S. Department of Agriculture for the yearly across-the-board commodity situation and outlook research. The State extension staff members supplement and adapt such research information to meet the commodity situation of their States.

Four States have projects that deal specifically with analysis of current price trends and prediction of future prices. There is increasing interest in longer range price prediction because of the growing specialization of farms, which make yearly enterprise shifts less common and less feasible, and which calls for large capital commitments over longer periods of time.

The total direct research effort in the situation and outlook area is approximately 1.7 professional man-years. While not designated as outlook research, much of the research conducted by the experiment stations and reported elsewhere contributes to improved understanding of price-making forces, which in turn improves market situation analysis and price fore-casting.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Livestock and Meat

In addition to the regular situation and outlook work, several special analyses were made. Attention was given to the cattle cycle which began its current buildup phase in 1959. Major factors considered were the length of the various cycles, the rate of buildup, and the effects of increased feedlot feeding on the present cycle. An analysis was made of average live weights of slaughter cattle and the relative price differentials between grades as steer and heifer beef production is increased. With the movement toward larger cattle feeding operations, an evaluation was made of the significance of changing seasonal placement patterns. Because of relatively low prices, particularly for fed cattle, special analyses were made of alternative USDA purchase programs and their effects on price and producer returns.

A study was made of U.S. foreign trade in livestock and livestock products, and results were published in the May issue of the <u>Livestock and Meat Situation</u> report.

Further attention was devoted to appraising the profitability of alternative feeding programs—short fed versus long fed. In addition, an analysis was made of the hog-corn price ratio through time. This analysis indicated that as labor and other costs advance the ratio is becoming a less reliable indicator of farrowings than in earlier years.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

- Hannawald, E. B. July 1964. Relation to hog slaughter to pig crops. Livestock and Meat Situation, pp. 28-33.
- Rockwell, George R. Jr. May 1964. U.S. foreign trade in livestock and livestock products. Livestock and Meat Situation, pp. 18-38.
- Thompson, John W. January 1964. Hide and leather situation brighter in 1964. Livestock and Meat Situation, pp. 26-28
- Thompson, John W. July 1964. Recent changes in hide marketings. Livestock and Meat Situation, pp. 34-37.
- Livestock and Meat Statistics, August 1964. Supplement for 1963 to ERS Statistical Bulletin No. 333, 162 pp.

SUPPLY, DEMAND AND PRICE OF AGRICULTURAL COMMODITIES Economic and Statistical Analysis Division, ERS

PROBLEM

Producers, processors, distributors and consumers need more accurate quantitative knowledge of the interrelationships among prices, production, and consumption of farm products. Farmers need to know the prices they may expect from different levels of production to plan for maximum returns. Cooperatives, processors, and distributors need adequate statistical information on price and consumption responses under different supply conditions to aid in distribution of agricultural supplies that lead to maximum returns to farmers. Similarly, Congress and the administrators of farm programs need to evaluate alternative proposals to modify existing price support and production control programs in terms of their impact on production, consumption and prices received by farmers. The development of new statistical methods and the application of existing methods for measuring the interrelationships among prices, production, and consumption of farm products serve these needs by strengthening outlook and situation work, providing the basis for special analyses of alternative agricultural policies, and assisting research workers in agricultural economics.

USDA AND COOPERATIVE PROGRAM

The program of basic research into the factors affecting prices, supply, and consumption of principal agricultural commodities has emphasized four broad research areas: (1) measurement of consumer response to price; (2) measurement of producer response to price and other factors; (3) measurement of the effect of supply and demand factors on prices to farmers and to consumers; and (4) improvement of statistical techniques for measuring agricultural economic relationships.

Changes in emphasis are made from time to time to utilize effectively the professional skills available and to adjust to work having the highest priority. The current emphasis is on a comprehensive analysis of the price-making forces in the feed-livestock economy, especially on factors affecting beef supply. As specific agricultural programs are usually proposed on a commodity basis, the current program is discussed in detail on a commodity basis though much of the actual research is carried on jointly for related commodity groups.

The USDA program of research in this area involves 6 professional man-years.

(a) <u>Livestock and Meat</u>. This work involves 1.0 professional man-year located in Washington, D.C. Research on livestock is part of a comprehensive analysis of the price-making forces in the feed-livestock economy. This study gives special attention to the quantitative measures that show what happens to the production of each commodity within the feed-livestock

sector following changes in price of one or more of the commodities. The study includes analyses for the United States as a whole and for regions to measure differences in price response and to allow for the important farm and non-farm alternatives available in each region.

PROGRAM OF STATE EXPERIMENT STATIONS

The States are engaged in intensive and extensive research in price analysis. Much of it is of a basic nature to gain an understanding of pricemaking forces. Most of this research is commodity oriented, though some projects are of a highly mathematical and theoretical nature aimed at improving price analysis methodology.

Research is being conducted to determine the demand functions for a large number of commodities in all the major commodity groups. This research will indicate the price elasticity, the income elasticity, and the cross elasticities of the commodities being studied. Some studies will show the effect of ethnic origin, race and various social factors such as urbanization and working wives on demand. There is increasing interest in the various components of demand at the higher and lower levels of the demand curve. This is being related to advanced pricing methods and to government programs such as marketing agreements and the stamp plan. Because researchers are finding that some changes in demand cannot be explained by price, income and supply of competing commodities there is increasing research interest in the basic motivational factors that determine eating and clothing habits.

The supply response to price changes is a matter that is receiving considerable attention. This is in part because of its significance to farm incomes and government programs. Significant progress is being made in understanding the relationship of the capital structure on farms to supply response and thus to the differences between long-run and short-run supply responses. Supply responses are also being studied from the standpoint of how and why farmers misjudge the market and how inertia also interferes with optimum resource allocation.

There are some projects to determine the changes taking place in the seasonal price pattern of commodities and how farmers can better utilize this information in making production and marketing decisions.

Some projects deal with the effect of quality differences on price and how the market could be improved to reflect prices more commensurate with the use, quality, or performance. Also there is interest in price prospects for new products arising from utilization research, and there is expanded interest in price relationships abroad.

The total State Experiment Station research effort in this area is approximately 53 professional man-years of which 11.8 is for livestock, meat and wool.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Livestock and Meat

Because of growing beef imports in 1963 and price pressures in the domestic cattle market, a special analysis was made to measure the relative importance of domestic fed beef production, cow beef production, beef imports, supplies of competing meats, and other factors on fed cattle and cow prices. In this study, the market for fed cattle and the market for cow beef were considered as two distinct but related markets. The analysis indicated that (1) a one-pound per capita change in fed beef production resulted in a change in the opposite direction of 50 cents in the Choice steer price at Chicago, (2) a one-pound change in the aggregate of cow beef production and beef imports changed the Choice steer price by about 30 cents in the opposite direction, and (3) most of the variation in fed cattle prices in the past two years could be explained by variation in domestic steer and heifer beef production. The study also showed the impact of beef and veal imports on cattle prices at different levels of imports. The results from this study are quite useful in making price forecasts and in appraising the effect of the government beef purchase program on fed cattle and cow prices.

Work on the price and supply of beef cattle has been concentrated on measuring growth in the beef inventory and the cycle in the beef inventory numbers. Growth rates for the period 1947 to date for numbers and value of the beef inventory, and for production of beef have been evaluated to serve as the basis for determining the necessary levels of herd placements that would be consistent with recent growth rates and possible rates in the future. relationship between slaughter weights of steers and the phase of the cattle cycle was studied. These findings were reported in a special article. A beef inventory model has been constructed and designed to predict the number of beef cows, steers, heifers, bulls and stags, and total beef cattle numbers six months in advance. Statistical results from this model, when completed, will be useful in making the annual outlook forecast each fall. A regional description of the location of basic beef cow herds has been completed, showing the relative concentration of the basic beef cow herds in the Southwest and Western United States in relation to other sections of the country.

Some progress has been made in developing a supply response model which treats separately the factors affecting the supply of fed beef and those affecting the supply of non-fed beef. Considerable time was spent in the development of data which would permit separate treatment of the fed beef and non-fed beef sectors. For example, regressions were used to estimate total liveweight of steers, heifers, and cows. This was then separated into the liveweight of fed beef and the liveweight of non-fed beef.

Work is also getting underway to improve quarterly forecasts of marketings and prices of fed cattle, prices of feeder animals, and the number of animals placed on feed. Some preliminary analyses have been made of the factors affecting the feeder market and the fed cattle market, and the interrelationships between these two markets.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Livestock and Meat

- Rojko, A. S. November 1963. The import situation for beef and veal. Livestock and Meat Situation, pp. 35-49.
- Walters, Forrest E. March 1964. The inventory cycle and slaughter weights of steers. Livestock and Meat Situation, pp. 25-28.



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BEEF CATTLE RESEARCH PROGRESS REPORT

Part I a of

Animal-Poultry and Products Research

A summary of current program and preliminary report of progress of the United States Department of Agriculture and related work of the State Agricultural Experiment Stations.

This progress report is primarily a research tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of research progress include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued during the past year. Current agricultural research findings are also published in the monthly U.S.D.A. publications, Agricultural Research and The Farm Index.

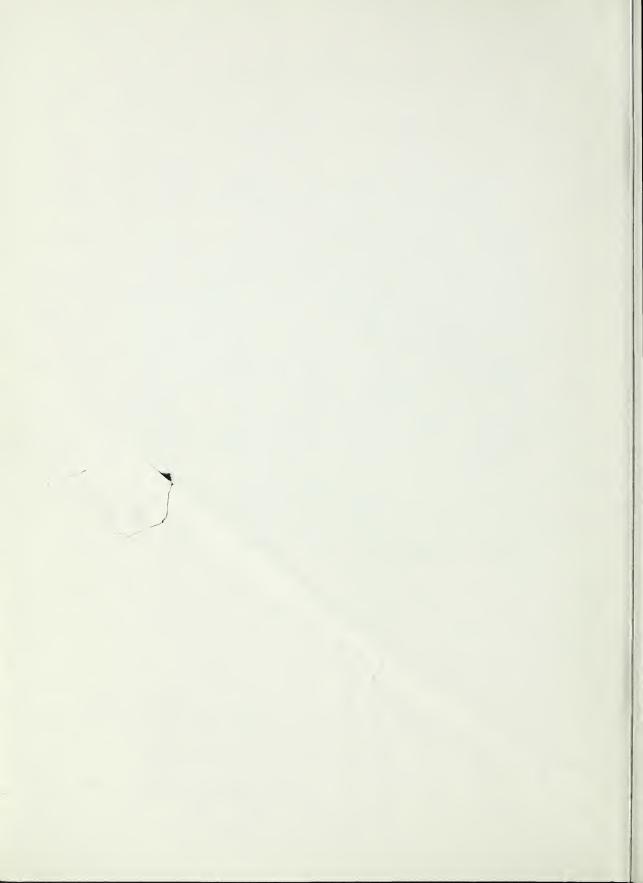
UNITED STATES DEPARTMENT OF AGRICULTURE Washington, D. C. 20250

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Part I-a, BEEF CATTLE RESEARCH PROGRESS REPORT

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The research program pertaining to beef is reported in two volumes: Part I-a and Part II of Animal-Poultry and Products Research. This volume contains a report of farm research that applies primarily to beef cattle, however, it is recognized that much of it could apply to dairy cattle as well. The companion volume Part II contains information that may be applicable to other classes of livestock.

The following subject matter progress reports are prepared by U.S.D.A. The number prefixes refer to advisory committees listed later that review the research reported:

- 6 Forestry (other than Forest Service)
- 7 Beef Cattle, Part I-a
- 7 Dairy, Part I-b
- 7 Poultry, Part I-c
- 7 Sheep and Wool, Part I-d
- 7 Swine, Part I-e
- 7 Animal-Poultry and Products, Part II
- 8 Cotton and Cottonseed
- 9 Grain and Forage Crops
- 10 Horticultural Crops
- 11 Oilseed and Peanut
- 11 Sugar
- 13 Tobacco

The information contained in the above subject matter reports was first reported in the following organizational unit reports. As above, the number prefixes refer to advisory committees listed later that review all of the work of the respective divisions or services.

Agricultural Research Service (ARS)

Economic Research Service (ERS)

- 1 Agricultural Engineering
- 1 Soil and Water Conservation
- 2 Utilization -- Eastern
- 2 Utilization -- Northern
- 2 Utilization -- Southern
- 2 Utilization -- Western
- 3 Human Nutrition
- 3 Clothing and Housing
- 3 Consumer and Food Economics
- 4 Market Quality
- 4 Transportation and Facilities
- 7 Animal Husbandry
- 7 Animal Disease and Parasite
- 12 Crops
- 12 Entomology

- 1, 5 Economic Development
- 4, 5 Marketing Economics
 - 5 Farm Production Economics
 - 5 Economic and Statistical Analysis
 - 5 Foreign Development and Trade
 - 5 Foreign Regional Analysis
 - 5 Natural Resource Economics
 - 6 Forest Service Research (FS)
- 4, 5 Farmer Cooperative Service (FCS)
- 4, 5 Statistical Reporting Service (SRS)

The research program of the Department of Agriculture is reviewed annually by the following advisory committees:

- 1. Farm Resources and Facilities Research
- 2. Utilization Research and Development
- 3. Human Nutrition and Consumer Use Research
- 4. Marketing Research
- 5. Agricultural Economics Research
- 6. Forestry Research
- 7. Animal and Animal Products Research
- 8. Cotton Research
- 9. Grain and Forage Crops Research
 - 10. Horticultural Crops Research
 - 11. Oilseed, Peanut and Sugar Crops Research
 - 12. Plant Science and Entomology Research
 - 13. Tobacco Research

A copy of any of the reports may be requested from Max Hinds, Executive Secretary, Animal and Animal Products Research Advisory Committee, Research Program Development and Evaluation Staff, U. S. Department of Agriculture, Washington, D. C. 20250

INTRODUCTION

The research program pertaining to beef reported in Part I-a and Part II, Animal-Poultry and Products Research Progress Reports covers work directly related to the production, processing, distribution, and consumption of beef cattle and resulting products. The information has been assembled from the organizational unit reports of the several divisions. These reports do not include extensive cross-commodity work, much of which is basic in character, which contributes to the solution of not only beef problems but also to the problems of other commodities. Progress on cross-commodity work is found in the organization's unit reports of the several divisions.

These reports are organized by "Problem Areas" which are shown in the table of contents. For each area there is a statement of (1) the problem, (2) the USDA program, (3) State experiment station programs, (4) a summary of progress during the past year on USDA, and cooperative work, and (5) a list of publications resulting from USDA and cooperative work.

Research on animal-poultry and products problems is supported by (1) Federal funds appropriated to the research agencies of the USDA, (2) Federal and State funds appropriated to the State agricultural experiment stations, and (3) private funds for research carried on in private laboratories or for support of State station and USDA work.

Research by USDA

Farm research pertaining to beef is conducted in the Agricultural Research Service divisions of Agricultural Engineering, Animal Disease and Parasite, Animal Husbandry, and Entomology. The work comprises investigations of breeding, physiology, nutrition, diseases, insects, housing and management, involving 152 professional man-years of scientific effort. This includes research on cattle diseases and parasites that is applicable also to dairy of which 29 man-years are devoted to domestic diseases, 20 to parasites, and 24 to foot-and-mouth disease.

Nutrition, consumer, and utilization research pertaining to beef is conducted in the Agricultural Research Service divisions of Human Nutrition, Consumer and Food Economics, and Eastern Utilization. The work comprises investigations of composition and nutritive value; physiological availability of nutrients and their effects; new and improved methods of preparation, preservation, and care in homes, eating establishments and institutions; and with the processing phase involving slaughtering the animals and processing the meat, tallow and hides. Also, it is concerned with improved equipment and processes. The work in these divisions applicable to beef is estimated at 90 professional man-years.

Marketing and economic research pertaining to beef is carried on within four services: Agricultural Research Service, Economic Research Service, Farmer Cooperative Service, and Statistical Reporting Service. The work comprises (1) physical and biological aspects of assembly, packaging, transporting, storing and distribution; (2) economic aspects of marketing costs, margins and efficiency, market potential, supply and demand, and situation and outlook; (3) cooperative marketing; and (4) consumer acceptance studies. The divisions in which the work is conducted are: Market Quality, ARS; Transportation and Facilities, ARS; Marketing Economics, ERS; Economic and Statistical Analysis, ERS; Marketing Division, FCS; Standards and Research, SRS. The work in these divisions applicable to beef is estimated at 22 professional man-years.

Interrelationships among Department, State and Private Research

A large part of the Department's research is cooperative with State Experiment Stations. Many Department employees are located at State Stations and use laboratory and office space close to or furnished by the Station. Cooperative work is jointly planned, frequently with the participation of representatives of the producers or industry affected. The nature of cooperation varies with each study. It is developed so as to fully utilize the personnel and other resources of the cooperators which frequently includes resources contributed by the interested producers or industry.

Including both cooperative and State Station projects beef research is carried on in 47 of the 53 State Experiment Stations. The types of work to which the largest amount of effort is devoted includes breeding, physiology, nutrition and management, diseases and parasites, marketing economics, and utilization research on meat and animal fats. There is regular exchange of information between Station and Department scientists to assure that the programs complement each other and to eliminate unnecessary duplication.

Privately supported beef research emphasizes the solution of scientific production, processing, and marketing problems. Much of it utilizes the results of basic work done by State Station and Department scientists.

About 1/3 of industry's contribution to the research effort pertains to farm research. In contrast with the poultry industry where practically all breeding research is done by industry, very little is done by industry in beef, except the work of large firms like the King Ranch which developed the Santa Gertrudis breed. The cope of operation required for a successful research program in breeding beef cattle, because of the size of animal and length of life cycle which tie up a substantial amount of funds, is undoubtedly a factor contributing to the amount of public research.

Another one-third of the research effort is in the utilization field. In contrast with the public research in basic work the industry program places strong emphasis on developmental activities and solving of immediate problems.

The work of meatpackers is devoted to finding industrial utilization of byproducts, quality control devices, improved formulation of products, improved
handling and plant arrangement. Independent laboratories and foundations
take on short time problem-solving for clients in the meat industry. Pharmaceutical firms carry on research on extraction of biologically active substances from meat by-products such as hormones from glands, and with the
development of agents, such as antibiotics for use in meat processing.

The contributions of beef producers and industry to the work of the State Stations and the Department have been an important factor in the success of their research programs. Producers offer herds and facilities for testing products and practices used in production. Likewise, processors and retailers offer facilities and products for use by public research agencies. Many problems in the economics of marketing cannot be transferred to a laboratory, experimental plot, or other simulated situation. The results of economic research conducted cooperatively is of great value to industry, especially in cases where public research can provide comparison and analysis. Even large firms that have a research staff do not have access to the plants and records of competitors.

Examples of Recent Research Accomplishments by USDA and Cooperating Scientists

All-grain rations can be used in cattle feeding. Steer feeding trials with all-concentrate finishing rations, based on corn, barley, milo, or wheat, demonstrated that: (1) cattle do not require roughage for health and efficient growth, (2) the addition of certain minerals found in roughages are of no benefit when added to the all-concentrate rations, (3) vegetable proteins are not superior to urea as a source of supplementary nitrogen, (4) wheat can be fed as the major source of carbohydrates, and (5) carcass quality is equal to that produced when roughage is fed. Studies also have shown that calves can be wintered satisfactorily by substituting barley for part or all of the hay.

Uniform blood typing results. A comparison testing program was established in 1956 by the USDA among cooperating blood typing laboratories. The original purpose was to improve repeatability of blood typing results from one laboratory to another. Twenty-two laboratories from 17 different countries participate. As a result of this coordinating effort, research results all over the world are repeatable and can be uniformly interpreted. An analysis of more than 75,000 individual tests of cattle red blood cells established that laboratory tests of duplicate cells produce the same results 99% of the time. New blood typing reagents developed in different parts of the world are quickly recognized. Agreement on identification nomenclature for several new blood factors has been obtained. Newly developing laboratories find the program particularly useful because they see how they "measure up" with the established laboratories.

Cow raised on PROTEIN-FREE ration gives birth to normal calf. An Angus heifer was raised on a protein-free, chemically pure ration. During the 26-month experiment, body weight increased from 290 to 930 lbs. The heifer matured sexually, conceived, and delivered a 51 lb. heifer calf while consuming this ration. This accomplishment provides a new research tool for the study of the nutritive requirements for cattle reproduction.

Studies with tissue culture-modified rinderpest virus as an immunizing agent. Through serial passage of a modified strain of rinderpest virus in tissue culture by the limiting dilution technique, an avirulent form of rinderpest virus resulted. This modified virus, when inoculated into cattle, resulted in a high degree of immunity and protection against the disease. Three weeks after immunization cattle challenged with 100,000 lethal doses of virulent rinderpest virus showed no signs of illness. Subsequent to this work, officials in Egypt requested this modified rinderpest virus immunizing agent for use in their country. Results to date have been very good in water buffalo, native Egyptian cattle and cattle imported into Egypt.

Pulmonary adenomatosis has been produced in cattle with the oxides of nitrogen. The pulmonary lesions produced by the inhalation of the oxides of nitrogen are similar to those observed in man affected with silo-fillers disease. Information from this research will be useful in explaining the pathogenesis of pulmonary alterations produced in both cattle and men exposed to these toxic gases. It is expected that this study will explain some of the alterations observed in bovine emphysema, bovine asthma, and fog disease.

Ultraviolet radiation and Moraxella bovis work together to cause bovine pink-eye. In a series of preliminary experiments, a mercury sunlamp was found to enhance the effect of Moraxella bovis infection upon the bovine eye. The resulting disease was indistinguishable from field cases of infectious bovine keratoconjunctivitis (pink-eye). This method makes possible the study of the disease under controlled conditions at any time of year. The investigators propose that ultraviolet has a primary etiological role in the disease.

Causal agent (Anaplasma marginale) of bovine anaplasmosis in the United States, comparable in virulence to supposedly benign form (Anaplasma centrale) in Africa. Bovine anaplasmosis, a costly disease, is caused by a microscopic parasite, Anaplasma marginale, located marginally in red blood cells. A related form, Anaplasma centrale, located centrally in red blood cells, occurs in African cattle. The latter is said to be harmless, and to protect against anaplasmosis caused by the "marginal" form. Critical comparisons of these two forms were made experimentally in Africa by a Beltsville scientist, using a "marginal" form transported to Africa, a "central" form procured there, and susceptible, imported cattle. Significant differences

between the two parasites were not detected, either in disease produced, including degree of anemia, or immunization ability. The findings of this evaluation are important because of strong recommendations that Anaplasma centrale be brought into the United States and used as a vaccine against anaplasmosis caused by the indigenous parasite. To do so could result in another disease agent becoming established in American cattle.

Ventilation of livestock buildings. Research in cooperation with State Experiment Stations has obtained much needed basic data on the heat and moisture given off by cattle, hogs, and poultry, and on the influence of building environment on production and feed consumption. The heat and moisture dissipation data are considered basic design data for ventilation systems of poultry, dairy, and swine buildings. They appear in design handbooks including the 1965 Guide and Data Book of the American Society of Heating, Refrigeration, Ventilating, and Air Conditioning Engineers, and are used by makers of ventilating equipment, prefabricated buildings and package buildings as well as by specialists advising farmers on their own construction. Building improvements resulting from the above research have contributed to the substantial rise in efficiency of livestock production that has occurred during the past decade.

Electrically Operated Gates Can Reduce Livestock Marketing Costs. Electrically operated gates that speed up handling of livestock through the sales ring and reduce labor requirements for the selling operation are being tested at a livestock auction market in Mexico, Mo. The auctioneer controls the movement of livestock into and out of the sales ring by pushbutton control of the gates, thus eliminating the need for workers in the ring to open and close the gates. Central control by the auctioneer of entrance, selling, and exit also eliminates some of the delays and mixups that occur under conventional handling methods.

Composition and Structure of Animal Fats Elucidated. Recent investigations have resulted in the development of unique techniques for determining the composition and structure of animal fats in minute samples of the fat. The procedure involves a hydrolytic breakdown of the fat glyceride by means of enzymes, separation of the hydrolytic products by chromatographic procedures, and conversion of the products to derivatives that permit their analysis by means of thin-layer chromatography. Because of the highly specific action of the enzyme in splitting only certain structures of the fat molecule, this method determines the nature of these structures with absolute certainty. This type of basic information, which is leading to a better understanding of fat composition and structure, is essential to further expansion of animal fat utilization in outlets as fats for improved shortenings and confectionery uses. In addition, because these techniques are particularly adaptable to very small samples, they are now finding wide application in the biomedical field for determining the glyceride composition of fats from tissue of small experimental animals, in studies of lipid metabolism as affected by certain diseases, and in plant genetics where it permits analysis of the fat in a single seed.

Perspiration-resistant Leather. A new tanning process yields leather with increased resistance to deterioration by perspiration, chemicals and washing. The process, based on tanning with glutaraldehyde, is in use by many tanners to produce improved shoe upper, insole, garment, and glove leathers from most types of hides or skins, such as cow, horse, sheep, pig, kangaroo and reptile. The process imparts other desirable properties to leather by increasing its receptivity to dyes, oils, finishes, water-repellent treatments, and other post-tanning operations. These improved properties will place leather in a better competitive position with respect to leather substitutes and should help to preserve this profitable market for animal hides. Estimates indicate that in 1964 over 40,000,000 square feet of leather were produced by the new tanning process. The largest volume use is for work shoes, where perspiration deterioration has always been a problem, but significant amounts are also used for casual and dress shoes.

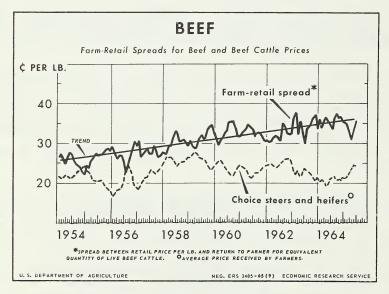
Mycotoxins Found To Be the Causative Agents of Fescue Foot. A number of toxigenic molds have been isolated from tall fescue. Their various toxic effects have been demonstrated in the mouse, rabbit and sheep by using suitable extracts of either the toxic hays or pure cultures of molds isolated from the hay. In the rabbit, topical application of extracts on the unabraded skin produces hyperemia, edema, hemmorhage and death. In the mouse, intraperitoneal injections of clear filtrates of submerged cultures of the molds produce death as a result of massive pulmonary and visceral hemorrhage. Force feeding of total mold cultures to a mature ewe produced total ruminal paralysis.

This latter result is comparable to the clinical pathology of cattle suffering fescue foot, in which death results from the ensuing starvation and dehydration. Three of the active metabolites of one of the toxic molds (Fusaria) have been isolated, and these are capable of producing the same toxic effects in rabbits and mice as the original hay or mold extract. These substances are being characterized chemically and evaluated in cooperative tests on cattle.

Some pesticides influence characteristics of food flavor. That flavor and eating quality of both meat and plant products may be affected by the use of pesticides during production has been shown by ARS food specialists in cooperation with other scientists. Rib cuts from beef animals sprayed with a pesticide used to control cattle grubs and horn flies had more off-flavor than those from untreated animals; the flavor of ground round, liver and kidney was not adversely affected. Off-flavors in potatoes grown in soil treated with the fungicide PCNB (pentachloronitrobenzene) varied with growing location and the level of PCNB used. Off-flavors were less evident after 3 to 4 months of storage. Information from these studies is used in developing Department recommendations for use of pesticides.

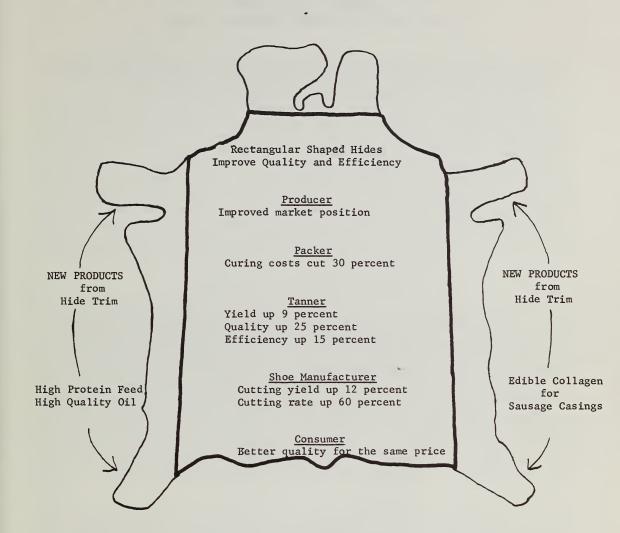
Forecasting the Beef Cattle Inventory. A special study was made to determine the position beef inventory numbers currently occupy in the cattle cycle. Based on historical relationships between cattle numbers and past prices, formulas were constructed to forecast the numbers in each of four classes of cattle and calves from the latest price information available. Forecasts can be made from six months to more than a year in advance; for example, forecasts of inventory numbers on January 1, 1967, could be made in late summer of 1965. Individual forecasts can be combined to form a forecast of the total beef cattle inventory. This study indicated that beef cow and calf numbers would drop when feeder calf prices fall and stay below \$21 for a sustained period of time.

Farm Retail Price Spreads. A keen interest in price spreads between farm and retail has existed for many years, especially by members of Congress and persons affected by the price spreads - farmers, processors, distributors, and consumers. The following chart illustrates graphically results of on-going research to help keep the public informed about economic conditions that can be used in formulating public policy and by industry in making its business decisions:



A remarkably persistent upward annual trend of about 1.1 cent per retail pound for choice grade beef prevails in the farm-retail spread. Deviations from the trend occur in periods when prices are falling or rising rapidly.

Improved Marketing Procedure for Hides. A study of the economics of modifying hide trims to meet competition and to provide shoemakers with hide leather products which most closely meet their raw material needs is reported graphically below:





I. FARM RESEARCH

BEEF CATTLE - BREEDING Animal Husbandry Research Division, ARS

Problem. Expression of each of the productive and carcass traits of beef cattle varies from breed to breed and between animals within each breed. The beef cattle producer is constantly striving to achieve excellence in one or more of these traits. Frequently his failure to choose the best animals for breeding stock for the most effective mating program results in less than maximum progress. Often the beef cattle producer does not know how to identify, evaluate, and utilize the existing variability to achieve his aim. Research information is needed on heritability of economic traits in beef cattle, genetic and phenotypic correlation between these traits, effectiveness of various selection and breeding programs, and assessment of traits most useful in beef cattle improvement. Basic information on cytology, inheritance of genetic abnormalities and genetic aspects of heritable biochemical and physiological characters will be required for a full understanding of applied problems.

USDA AND COOPERATIVE PROGRAM

The beef cattle breeding research in the United States has developed as a coordinated program of the USDA and the State experiment stations. It is a continuing program of both applied and basic research carried on by geneticists, animal physiologists, and animal husbandmen. Early efforts in the improvement of beef cattle through performance testing were made by the USDA at Miles City, Mont., and Beltsville, Md. With the advent of regional research, efforts by the State stations were greatly increased and the individual programs were coordinated through regional research projects in three of the important beef cattle producing regions. This joint activity has been and remains characteristic of beef cattle breeding research, and the resulting program is an integrated effort combining to the best advantage the resources of the State experiment stations and the USDA.

The regional project in the South is S-10, Improvement of Beef Cattle for the Southern Region through Breeding Methods. Much of this region is subtropical in climate and in many cases cattle used in other areas are poorly adapted. Environmental conditions adversely affecting survival, reproductive regularity and growth are encountered. Research includes active projects at 12 State stations and at the USDA stations at Jeanerette, La., Front Royal, Va., and Brooksville, Fla.

In the Western region the beef industry is largely geared to range conditions with many cattle shipped to areas of abundant grain supply for fattening. Ability to make maximum use of forage available on the range is an important consideration. These problems are studied through regional

project W-1, The Improvement of Beef Cattle through the Application of Breeding Methods. Research includes projects at 12 State stations and at the USDA station at Miles City, Mont.

Similarly, NC-1, Improvement of Beef Cattle through Breeding Methods, is geared to problems of the beef industry in the North Central region where beef is produced on farms with pastures of high productivity and ample grain supplies for feedlot finishing. Research includes projects at 12 State stations and at the USDA stations at Fort Robinson, Nebr., and Fort Reno, Okla.

The Federal scientific effort devoted to research in this area totals 15.7 professional man-years. Of this number, 1.3 are devoted to performance testing, 5.8 to genetics and interrelationships of performance traits, 1.7 to genetic-environmental interactions, and 6.9 to selection and systems of breeding.

PROGRAM OF STATE EXPERIMENT STATIONS

To a greater degree than many other research programs, beef cattle breeding research has developed as a coordinated program of the USDA and the State agricultural experiment stations. This has been achieved to a large degree by cooperative research activities under three regional beef cattle breeding projects. This coordinated program is described amply by the material appearing under the USDA and Cooperative Program. The several examples cited describe research of a cooperative nature, much of it conducted at the State experiment station locations. The reader is referred to this section of the Division report with these comments, and no attempt will be made to summarize the State programs separately.

The total research effort on beef cattle breeding research by the State agricultural experiment stations is 65.0 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

As compared to other disciplines, knowledge accumulates slowly in beef cattle breeding and genetics due to (1) low reproductive rates, and (2) high maintenance and operational costs per animal; i.e., per genetic unit. In the material which follows an attempt has been made to include only items on which specific analyses were completed or summaries made during the year.

A. <u>Selection and Systems of Breeding</u>

1. Effectiveness of selection for economically important traits. In Colorado, a study was completed on data from 14 Hereford inbred lines developed over a period of 18 years. The level of inbreeding increased rapidly in the formation of these lines and averaged 30% over the entire period. Estimates of genetic change were positive for feed efficiency and

negative for weaning weight and score in both sexes; and for final weight off test, average daily gain, and final grade among bulls. The level and rate of change of inbreeding appeared to have been real factors in the response of individual inbred lines to selection. (AH d1-16)

In a similar study at the Oregon Station involving one Angus and three Hereford lines, selection practiced for economic traits was positive for all traits studied. Automatic selection against inbreeding occurred on the sire side in conjunction with selection for increased performance. In general, performance increased early in the program, then leveled off, and subsequently declined. General improvement in all traits occurred only in the Angus line. (AH dl-19)

In a Nebraska experiment that is in the first generation of selection for single traits, foundation sires and their sons, selected on the basis of specific criteria, have sired calves in the same year. The selected sons produced progeny that were generally superior to the progeny of their sires in the primary selection criteria. Furthermore, the foundation sires were a highly select group. (AH d1-12)

2. <u>Crossbreeding</u>. Comprehensive analyses were made of the data from an extensive Fort Robinson, Nebr., crossbreeding experiment including the Hereford, Angus, and Shorthorn breeds. In the first phase of this experiment the three straightbreds and all reciprocal crosses among them were produced. Crossbreds and straightbreds were sired by the same bulls and were out of comparable cows. These studies included a total of 751 cattle from four calf crops sired by 16 Hereford, 17 Angus, and 16 Shorthorn bulls.

The effects of hybrid vigor were significant for most of the economic traits evaluated. A 3% greater calf crop was weaned in the crossbred than in the straightbred calves because of differences in early postnatal mortality. The heterosis effect on postweaning growth rate of heifers on a low level of feeding was greater than in steers on a growing-fattening ration. Heterosis tended to decrease with increasing age after approximately one year and was greatest on a restricted feed intake when comparing heifers with steers. After adjusting age at puberty for the effects of average preweaning and postweaning daily gains, approximately one-half to three-fourths of the heterosis effect on age at puberty (days) remained. Thus, there was a heterosis effect on age at puberty independent of its effect through average daily gains. The advantage of the crossbred steers in feed efficiency was small.

In net merit (value of the boneless, closely trimmed retail meat, adjusted for quality grade, minus feed costs from weaning to slaughter) the advantage of the crossbred steers over the straightbred steers was \$8.81 per carcass. This net merit difference is among the steers that lived to slaughter. The 3% advantage for the crossbreds in calf crop weaned was not involved in computing this difference.

For growth, feed efficiency, and carcass traits the heterosis effect was greater in the Hereford-Angus and Hereford-Shorthorn combinations than for the Angus-Shorthorn combination, while for age and weight at puberty, the heterosis effect was greatest for the Hereford x Shorthorn and reciprocal cross. In evaluating all traits for the effects of heterosis, it can be concluded that heterosis results in an increased rate of maturity.

The second phase of this experiment is now in progress. This involves the evaluation of the effects of hybrid vigor on fertility and mothering ability. That is, straightbred cows of the three breeds are being compared to their crossbred half-sibs when both are bred to the same bulls. For the two years (1963 and 1964) on which data have been collected, the advantage of the crossbred cows has been 17 and 6%, respectively, for calf crop weaned and 17 and 31 lb., respectively, in average weaning weight of calves at 200 days.

Milk production studies showed that crossbred cows gave no more milk than straightbreds immediately after calving but gave approximately 10% more milk in mid-lactation. (AH d1-12)

Ohio studies involving Charolais x Hereford and the reciprocal cross compared with their straightbred half-sibs from the parental breeds show a heterosis effect on growth rate.

Charolais, Brown Swiss, and Holstein topcrossed on Hereford have produced cattle with faster gains than the straightbred Herefords. However, the design of this experiment does not lend itself to an evaluation of the heterosis effects. (AH d1-10)

In Louisiana studies, crossbred calves grew somewhat more rapidly than purebred calves throughout the entire preweaning period. The preweaning growth curve was different for calves from British breed cows, as compared with calves from Brahman and Brangus cows. Calves from all four breeds of dam-Angus, Brahman, Brangus, and Hereford - grew at about the same rate for the first 140 days. At that time, growth rate of calves from British breed cows decreased while the growth rate of calves from Brahman and Brangus cows remained relatively constant. This may be due to greater persistency of lactation in Brahman-type cows. These data support the previous evidence that the effect of age of calf on weaning weight varies considerably from one breed to another.

Additional information on age of puberty in straightbred and crossbred heifers in this study indicates that breed crossing results in an appreciable amount of heterosis for this trait.

Also, at the Louisiana Station, a summary of calving dates and calving percentages by various sire-dam breed combinations indicates that Brahman bulls show some degree of selectivity as to the kind of cows they mate with.

Observation of the breeding behavior of Brahman bulls over the past 12 years has brought out the fact that some Brahman bulls definitely show strong preference for Brahman-type cows.

Several stations have been comparing different systems of mating for several years. The Georgia Station has completed one generation of a comparison of grading-up, crisscrossing, and three-breed rotational crossing. There has been little difference in the three systems in percentage of calf crop born, but due to a higher survival rate among both types of crossbreds, they have had a 3% greater net calf crop than the straightbred calves. (AH d1-3)

Another study comparing two-breed crosses with three-breed crosses indicates an advantage at weaning time of approximately 23 pounds in favor of the three-way cross steers. However, no differences were noted in postweaning performance. Smaller differences were obtained in heifers than in steers. A separate study showed a significant difference between breed crosses for weaning weight in favor of the crossbred calves. The crossbred cows in this study weaned a higher percentage of calf crop and remained in production longer than did straightbred Hereford controls.

The preliminary Miles City data indicate no consistent evidence of hybrid vigor for age at puberty in crossbred heifers as compared to straightbred heifers of the Hereford, Angus, and Charolais breeds. Data on bulls showed evidence of hybrid vigor in age at puberty for all breed crosses. There is evidence of hybrid vigor for birth and weaning weights among steer calves with the crosses exceeding the average of the parental breeds by 29 to 44 lb. in weaning weight. Crossbred steers also indicate substantial advantage over straightbreds in feedlot gain and feed efficiency. However, there is little evidence of hybrid vigor in preweaning or postweaning gain among the heifers. (AH dl-1)

The California data continue to indicate hybrid vigor for weights and gains but very little or no hybrid vigor for live and carcass grades or dressing percent. Based on palpation two months after the breeding season, the Angus x Hereford, Angus x Shorthorn, and Hereford x Shorthorn crosses indicated hybrid vigor of 10, 13, and 4%, respectively, for conception rates. (AH d1-40)

Facilities have been completed at a new experiment station in Hawaii and a crossbreeding study involving the Hereford, Angus, and Charolais breeds was begun during the 1965 breeding season.

3. <u>Inbreeding and linecrossing</u>. Adding to and, in general, confirming trends indicated in previous reports, studies on the effects of inbreeding on performance traits have recently been completed at Colorado, Oregon, New Mexico, Wyoming, and the U. S. Range Livestock Experiment Station, Miles City, Mont. It appears evident that increased inbreeding is associated with decreases in growth and live scores or grades. In general, the detrimental effects of inbreeding tend to decrease with increased maturity. However,

the magnitude and duration of the inbreeding effect appear to vary widely with the breed, line, location, sex, and level of environment. Inbreeding of dam has a detrimental effect on preweaning growth of calves and in many cases the effect is greater than the inbreeding of calf effect. (AH dl=16, AH dl-19, AH dl-2)

Preliminary results from the Miles City linecrossing study involving five inbred lines indicate that linecross bull calves have a 3% advantage in birth weight, 6% in 180-day weaning weight, and 3% in weaning score over contemporary inbred calves. The linecross bulls gained 3% faster in the feedlot and weighed 4% more at the end of the test. The linecross heifers weighed 3% more at birth, 9% more at weaning, scored 3% higher at weaning, and weighed 10% more at 18 months of age. Inbred line performance was indicative of performance in the crosses. (AH d1-2)

In a preliminary analysis of a similar study at Oregon, linecross calves (inbred dams) gained 0.09 and 0.12 of a pound per day faster, respectively, in the preweaning and postweaning periods than contemporary inbred calves. Linecross calves required 27 pounds less feed per 100 pounds gain than inbred calves. (AH d1-19)

The Arizona Station has initiated a project involving the testing of ten inbred Hereford lines developed at seven Western experiment stations. Information on topcrossing and general combining ability of the lines will be obtained and compared to controls produced by industry bulls on the same cow herd. (AH d1-46)

Preliminary results from crossing South Dakota lines of the Hereford breed with low levels of inbreeding indicate that an appreciable amount of heterosis may be obtained from specific crosses. The performance of some of these lines in top cross evaluations, when compared to outbred stocks, indicates that within line selection has been effective in improving traits that have an appreciable amount of additive genetic variation. (AH d1-13)

Comparison of inbred lines within breeds and between breeds - as well as to lines selected for type and growth - continues at the Front Royal Station. This project has proceeded far enough that bulls from the inbred lines, as well as the selection lines, will be topcrossed on unrelated cattle at Blacksburg, Virginia, for the Shorthorn lines and at Mississippi State University for the Angus lines. (AH d1-4)

B. Performance Testing

1. Carcass traits. Research on methods of estimating potential carcass quality in live animals has continued. In all three regions, use of ultrasonic devices on live animals has continued to give reasonably good estimates of carcass fat thickness. There are, however, differences among correlations from different groups of cattle. (AH d1-1, d1-9, d1-13, d1-36)

Utah has accumulated information on various methods of estimating the amount of fat in live animals. Tritium and N-acetyl-4 amino antipyrine are both quite effective, with tritium being the more accurate. These methods are very technical and require considerable equipment.

In a study evaluating the accuracy of predicting carcass composition, a multiple correlation equation containing the variables of carcass weight, rib eye area, single fat thickness at 12th rib, untrimmed round weight, edible portion round weight, and pelvic and kidney fat weight accounted for 94% of the variance in edible portion. Omitting the information on the round reduced this to 89% and the further removal of pelvic and kidney fat weight reduced it to 86%. Using carcass weight, rib eye area, and the average of three fat thickness measurements at the 12th rib accounted for 84% of the variability. Carcass weight alone accounted for 79%. (AHd1-13)

An analysis of data from the Nebraska crossbreeding experiment indicated an appreciable amount of additive genetic variation on carcass composition on a weight constant basis. This is not consistent with earlier preliminary findings which indicated that adjustment of carcass composition for weight effects removed most of the additive genetic variation in carcass composition. (AH d1-12)

In Michigan studies the relationship among carcass traits on data collected during the year appears to support the findings of previous years. Loin eye area was not a good indicator of percent preferred cuts (r = -.27). When carcass weight was held constant, the standard partial regression of percent preferred cuts on loin eye area was 0.19. The correlation between percent preferred cuts and carcass weight was -.68.

There continues to be a slight negative phenotypic correlation between adjusted percent preferred cuts and tenderness index (r = -.24).

For each of the last three years, the correlations between pounds of preferred cuts and carcass weight were above 0.95. Thus, selecting for weight of preferred cuts would be essentially synonymous with selecting for growth rate.

The correlation between percent round in the two halves was 0.88 and between percent loin in the two halves 0.65.

Live animal estimates of loin eye area and percent preferred cuts for two years have been analyzed. The appraisers estimated loin eye area more accurately than percent preferred cuts.

Carcass data on steers were studied to identify carcass attributes which contributed variation to carcass retail value. It is quite clear that most cattle can produce beef of satisfactory quality if a satisfactory method of feeding and management and length of feeding period are provided. In experiments where an effort was made to market the cattle as they reached

suitable market finish, variation in carcass weight was the dominant attribute in determining carcass value. Fat trim, the next in importance, ranked far below carcass weight. Weight of round (carcass weight constant) was the most useful of the wholesale cuts as an indicator of carcass value. (AH dl-10)

Several studies on production and carcass traits of bulls and steers have confirmed earlier results to the effect that young bulls excel steers in growth rate, feed efficiency and leanness of the carcass. Certain muscles from the steer carcasses were somewhat more tender when measured by either taste panels or the Warner-Bratzler shear. The steer carcasses had an advantage in carcass grade. (AH d1-9, AH d1-12, AH d1-32, AH d1-36, AH d1-40)

2. Growth rates. The Arkansas station has studied the relative association of feed consumption, initial weight, and indicators of carcass leanness with feedlot gains. A comparison of multiple correlations indicates that about 16% of the variance in gain was associated with round and loin weight, ribeye area, and fat thickness. On a constant weight basis, forequarter and chuck were significantly related to gain (r = 0.33).

Data on plasma cholesterol concentration were summarized by the Arizona station at four stages of development of range Herefords. Environmental conditions associated with year of birth may exert a significant effect upon cholesterol concentration in blood. No effect of age of dam was detected, and age of animal showed no consistent effect on concentration. There was no apparent relationship between plasma cholesterol concentration with subsequent concentrations or with growth traits. (AH dl-46)

Hematocrit values, total serum protein, percent serum albumin and globulins have been determined on yearling bulls by the Wyoming Station. Line differences were found in all variables except beta globulin, indicating possible genetic differences. However, none of the traits was related to performance up to yearling age.

Oregon reported that levels of enzyme activity between slowly and rapidly growing animals or between younger and older animals indicated that the animal's metabolism may be related to levels of enzyme activity. (AH d1-19)

Data were analyzed on 100-day and 205-day calf weights for calves raised on creep feeders and without creep feeders. The early weights appear to be good indicators of dam's producing ability and they also reflect weaning weight of the calves accurately. Correlations between age adjusted weights and average daily gains are so high that either contains practically all of the information on the growth of the calves. The phenotypic correlations between first weight and weaning weight for individual calves were 0.75 for creep fed calves and 0.82 for non-creep calves. The correlations between dam's producing ability as expressed in early weights and later gains of consecutive calves was 0.75 for creep fed calves and 0.66 for non-creep calves. (AH d1-10)

Postweaning growth records of steers indicate that initial weight and initial age were not important sources of variation for later gains unless the original variation in weight and age were quite large. Correlations between gains made in 28-day periods and total gain indicate gains in early periods tend to be slightly better indicators of total gain than those made in later periods. (AH d1-10)

One recently completed study reports correlation coefficients of preweaning average daily gain with weaning weight, 18-month weight, and 24-month weight of 0.82, 0.59, and 0.57, respectively, for Brangus and 0.82, 0.37, and 0.31, respectively, for Africander-Angus replacement heifers. The relationship of weaning weight to 18-month weight was 0.76, and the relationship to 24-month weight was 0.62 for Brangus and 0.88 and 0.78, respectively, for Africander-Angus. Preweaning average daily gain of steers - limited fed during the winter and then placed on postweaning feeding test - was significantly related to postweaning gain on test, lifetime gain, and final weight in both Brangus and Africander-Angus. (AH d1-6)

In work relating to feed consumption and other economic traits, the Arkansas Station reports that about 59% of the variance in gain is associated with feed consumption and initial weight. In this same study, bulls with heavier testicles were poorer converters of feed when put on a constant weight basis (r=0.44); whereas, the bulls with more lean tissue - as indicated by cut weights and muscle area - were better converters of feed. In the same study, agonistic behavior and social rank indicated no breed differences in agonistic behavior. Chest depth and heart girth were significantly correlated with agonistic behavior within a group.

3. Cow size. Several S-10 studies indicate that cow size is heritable and may be related to production. Analysis of data from the North Carolina Station indicated that cows which were heavier 90 days before calving tended to produce heavier calves at birth and throughout the suckling period. These data suggest that this association is larger for younger cows and that the pattern of change in dam's weight appeared to be subject to herd and age effects. In the youngest class of dams, cows producing the faster-gaining calves showed smaller weight loss during the last 90 days of gestation and the first 60 days of lactation and higher gains throughout the remaining part of the lactating period. Older cows producing faster-gaining calves tended to have greater losses during the first 60 days of lactation and to gain more thereafter. (AH d1-23)

The effect of weight of dam on 180-day weight of Hereford calves raised at the Texas Station and on weaning weights of Angus calves raised in a privately-owned herd was studied. Hereford cows were weighed at calving and the Angus cows weighed at weaning. A linear relationship between calf weight and cow weight was observed in the Angus. A curvilinear relationship was observed in the Herefords.

An analysis of cow weight records from herds in Alabama, Florida, Georgia, Louisiana, North Carolina, South Carolina, and Texas showed all sources of variation, including location-year, breed within location-year, sire within sire, progeny within sire, age of dam and previous parity plus calving month for weight at calving, were statistically significant at the 0.05 level. These data indicated that cow weight increased with each year's increase in age up to nine years of age - the limit of these data - but the rate of increase was less with age. (AH d1-44)

- 4. <u>Maintenance</u>. On full feeding heifers consumed more energy per unit of metabolic weight than bulls. Efficiency of energy use by bulls and heifers appeared to be similar. However, heifers stored 29.2% of the net energy consumed and bulls 21.9%. This difference was apparently due to appetite. Correlation coefficients between live weight and carcass calories were 0.994 and 0.977 for heifers and bulls, respectively.
- 5. Selection indexes. Theoretical studies of probable effectiveness of selection for total net merit and for post-weaning net merit were made using single-trait and index selection. It appeared that selecting for final weight alone (452 days of age) would be 90% as effective in maximizing profit as selection for an index including pre- and post-weaning growth, feed efficiency and fat thickness (an indicator of carcass composition). Selection for weaning weight alone should be about 80% as efficient as selection for the index.

C. Genetics and Interrelationships of Performance Traits

1. Heritability of some performance traits. From an analyses of data (480 animals), estimates of heritability for some economic traits were as follows: average daily TDN consumption 0.46, percent retail product (boneless, closely trimmed meat from the entire carcass) 0.24, weight of retail product adjusted for age 0.65, fat thickness at 12th rib 0.50, quality grade of carcass 0.32, and net merit (value of retail product, adjusted for carcass quality grade, minus feed costs from weaning to slaughter) 0.55. (AH d1-12)

Heritability estimates were determined for growth and conformation traits for bulls and heifers in two lines of Herefords and for carcass traits of 80 randomly selected bulls. Low heritabilities were found for birth weight (5%), final conformation score (2 to 7%), final index (weight and conformation score) (15 to 24%), carcass conformation score of bulls (0.0), marbling score of bulls (0.0) and carcass grade of bulls (0.0). These estimates are not consistent with other studies for these traits. Medium heritability estimates were found for average daily gains of heifers (39%), left side carcass weight of bulls (40%), fat thickness of bulls (36%), trimmed primal cuts of bulls (32%), trimmed wholesale cuts of bulls (39%), and retail yield of bulls (42%). Higher than usual heritability estimates were found for average daily preweaning gains (89%), 210-day weight (80%) and

210-day weight adjusted for age of dam (65%). Ribeye area of bulls (72%), and total trimmed fat of bulls (54%) were found to be highly heritable. (AH d1-42)

A study on predicting the producing ability of range cows using weights and gains taken throughout life was reported by the Miles City station. Heritability of producing ability was estimated to be 0.45 based on an average of 3.2 calves per cow. Phenotypic, genetic, and environmental correlations of weights and gains from birth through maturity with producing ability were small and were not accurate indicators of producing ability. Eighteen-month weight was the best single predictor of producing ability both genetically and phenotypically. (AH d1-1)

Data from a Louisiana study showed large although not significant differences in calving percent between breeds and crosses.

The Florida station has found heritability estimates of calving percentage to be in the neighborhood of 0.3 to 0.6, considerably higher than previously reported.

2. <u>Genetic correlations</u>. Data from 480 animals show high positive genetic correlations between different periods of growth and weights at different ages. The correlations reveal that genetic improvement in <u>percent</u> retail product will likely be difficult if quality grade is improved or maintained. Also, these results indicate that percent retail product is of questionable value as a selection objective. (AH d1-12)

Results of heritability and genetic correlation studies of carcass data indicate that selection for growth rate would result in a greater increase in yield of separable lean than would selection for composition itself due to the high genetic correlation between lean and growth and the high heritability of growth. (AH d1-13)

- 3. Genetic-environmental interactions. Work on genetic-environmental interactions is being continued in two studies one a cooperative effort between the Stations at Brooksville, Florida, and Miles City, Montana; the other at the North Carolina Station. Data at the North Carolina Station indicate that sire-location interactions or evidence of genotype-environmental interactions are small. There was a large contrast this year between the calving percentages of the Miles City cattle transported to Florida, as compared to cattle of the Miles City line raised at Florida and the Hereford cattle that have been at Brooksville for several generations. (AH d1-41), (AH d1-23)
- 4. <u>Dual-purpose cattle breeding</u>. A herd of Milking Shorthorn cows (not part of any regional breeding project) has been maintained under a cooperative arrangement with the Minnesota Agricultural Experiment Station with the general objectives of (1) improving genetic capacity for milk

production as rapidly as possible, and (2) observing changes, if any, in beef production qualities of male calves as genetic milk production level of the herd increases. To this end, cows have been inseminated with semen of progeny-tested bulls carrying the highest apparent genetic milk production potential available either in the United States or abroad. Steer calves have been fed out with Holstein and Herefords and their carcasses evaluated.

Since 1959 the rolling Dairy Herd Improvement Association herd average has fluctuated somewhat but has shown a rather consistent rise from a low of 5,715 lb. of milk and 222 lb. of butterfat to its present level of 9,077 lb. milk and 341 lb. butterfat. Eighteen daughters of the two best bulls used (both New Zealand animals) have freshened and preliminary information shows an apparent superiority of approximately 2,100 lb. milk and 60 lb. butterfat for their daughters over herdmates.

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BEEF CATTLE - PHYSIOLOGY Animal Husbandry Research Division, ARS

Problem. Reproductive performance is the largest single factor affecting net profit in a cow-calf operation. Increasing the reproductive performance of a beef cow herd will result in more pounds of calf weaned per cow bred. More cows will wean a calf, and more cows will calve early in the calving season when reproductive performance is improved. Poor reproductive performance is largely the result of: (1) cows not showing heat early in the breeding season, (2) cows not conceiving at first service, and (3) calf losses occurring at or near calving. The physiological mechanism responsible for the onset of estrus and conception must be explored further and methods of controlling these adequately must be found if reproductive performance is to be improved.

USDA AND COOPERATIVE PROGRAM

The program at present is concerned with methods of altering, improving, or controlling reproductive performance by hormonal and nutritional methods and determining basic information on hormonal relationships during the estrous cycle. The program has approximately 144 cattle involved at Jeanerette, La.; 500 at Fort Robinson, Nebr.; 110 at Miles City, Mont.; 140 at El Reno, Okla.; and 136 at Beltsville, Md. Some reproductive information is also obtained on cattle involved in other studies at all of these locations except Beltsville. Major studies involve: (1) relationship between nutrition and reproductive performance at Miles City, Fort Robinson, El Reno, and Beltsville; (2) hormonal control of the estrous cycle at Fort Robinson; (3) causes of maintenance and regression of the corpus luteum at Fort Robinson; (4) induction of twin ovulations at Miles City; and (5) causes of calf losses at or near calving at Miles City and Fort Robinson.

The Federal scientific effort devoted to this area of work totals 3.1 professional man-years, all of which are devoted to physiology of reproduction.

PROGRAM OF STATE EXPERIMENT STATIONS

Research on physiological problems related to beef cattle production is conducted at many stations. These studies include the effect of controlled temperature and hormones on reproduction in heifers of both Brahman and British breeding, the nature of sterility in animals which leave herds because of failure to reproduce, and research designed to more clearly define fundamental principles related to ova transfer and to develop a technique for collection and transfer of ova without surgery. Investigation of physiological effects of various hormone substances, and development of simplified methods for bringing groups of animals into estrus within a short period is the objective of other research. Basic studies will determine the site of maturation of sperm, and if proven to take place in the uterus, attempts will be made to isolate the material responsible for maturation. A portion of this

work is conducted under the W-49 regional project "Physiological Mechanisms Affecting Fertility in Cattle."

Research in environmental physiology includes developing biological measures of response to environmental stress under controlled conditions, procedures for measuring environmental responses under field conditions, and the effect of nutrient restriction following weaning on the growth of heifers and upon subsequent lifetime production. A portion of this work is conducted under W-46 regional project "The Effects of Environmental Stresses on Range Cattle and Sheep Production."

Other studies seek to explain the action of hormone compounds in promoting growth and the effect of levels of milk and forage intake at different periods on gain and weaning weight of beef calves.

The total State effort devoted to physiology of beef cattle is 20.5 professional man-years. An additional 5.0 professional man-years pertains to Area 1 and is included therein.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Physiology of Reproduction

1. Nutrition and reproduction. Three experiments are underway in drylots. Calving difficulty was increased in heifers fed moderate levels of TDN (8.0 lb.) compared to heifers fed low levels of TDN (4.3 lb.). However, no difference in losses at calving were noted. The length of the interval from calving to first heat was longer in cows on low levels of feed prior to calving. However, the proportion of cows that became pregnant in a 60-day breeding season did not differ. Feeding cows high levels of feed after calving did not appear to improve reproductive performance. This is in contrast to previous years' results. (AH d1-37, AH d2-22)

The influence of various levels of supplemental range feeding on reproductive performance is being studied at four stations. Results indicate that the nutritional level supplied by pastures in the Gulf Coast region does not support adequate reproductive performance. Low levels of winter supplementation in Oklahoma led to a longer interval from calving to first heat. Data from Fort Robinson indicate that cows supplemented after calving returned to heat much sooner than cows not supplemented. Data from Miles City indicate little difference in reproductive performance between heifers receiving 8 pounds of supplement and those receiving no supplement. Thus, results vary from area to area.

Two studies have been conducted regarding the effect of nutritional level on age at puberty. In Louisiana and Nebraska the low level of nutrition delayed the onset of puberty in straightbred heifers more than in crossbreds. Little or no difference in puberty was noted between different breeds of heifers on high level of feed at Nebraska but Brahman heifers had delayed puberty in

Louisiana even on high levels of feed. (AH d1-37, AH d2-12, AH d1-33, AH d2-34)

Studies conducted at Front Royal, Va., and Brooksville, Fla., have not shown beneficial effects on calf survival or subsequent conception rate from supplemental vitamin A feeding or injecting to normally managed pregnant beef cows. (AH d3-2, AH d1-4).

- 2. Losses at or near calving. Most calf death losses occur at or near birth. Many of the losses are the result of delayed or difficult parturition. Losses have been higher in straightbred than crossbred calves. (AH d1-30, AH d1-33, AH d1-37)
- 3. <u>Puberty</u>. Results are available from Jeanerette, Miles City, and Fort Robinson. Results from Fort Robinson indicate that crossbred heifers reach puberty at an earlier age while data from Miles City fail to show a heterotic effect on age at puberty. Age at puberty in bulls showed a small difference in favor of crossbred bulls. (AH dl-33, AH dl-37, AH d2-34)
- 4. Control of estrus. In a series of trials it was demonstrated that estrus could be successfully synchronized by feeding a new synthetic progesterone-like compound for nine days, followed by a small injection of a synthetic estrogen. Results indicate that cows suckling calves can be successfully synchronized by this treatment if they are cycling prior to start of treatment. Fertilization rate, length of heat, and time of ovulation in heifers synchronized by this method did not differ from control heifers. (AH d1-37)

Causes of maintenance of the corpus luteum have been explored further this past year. The regression of the corpus luteum noted following small, daily injections of estradiol 17-beta were reversed by injection of HCG. However, equine LH, FSH, or a combination of the two were ineffective in this regard. Daily injections of 500 I.U. of HCG also maintained the corpus luteum to the 24th day of the estrous cycle. (AH dl-37)

5. Fertility data from breeding herds. Data from Fort Robinson and Jeanerette indicate that reproductive efficiency is increased in crossbred cows. More crossbred than straightbred cows settled on first service and more crossbred cows became pregnant. The interval from calving to first heat did not differ between straightbred and crossbred cows at Fort Robinson but did at Jeanerette. Breed of sire has also been shown to affect pregnancy rate at Jeanerette with Angus bulls having a higher pregnancy rate than Brahman bulls. (AH d1-30, AH d1-37)

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BEEF CATTLE - NUTRITION AND MANAGEMENT Animal Husbandry Research Division, ARS

Problem. Producers of beef cattle need improved feeding methods which will result in optimum pasture and feedlot gains, reduced feed consumption per pound of beef produced, optimum reproductive rates, and desired carcass qualities. To meet these needs basic nutritional information is required such as: When should beef animals be fed for maximum gains and when for more limited gains? What nutrient combinations produce rapid growth of muscle with a minimum of fat deposition? How may breeding animals be economically raised that will be capable of a high level of reproductive performance over a long lifetime? What are the nutritive contributions made by range and pasture and what supplementation is required when each is used? Research is also needed on the relationship between animal production and type of shelters and equipment, feeding systems, and methods of increasing labor efficiency.

USDA AND COOPERATIVE PROGRAM

This is a continuing program carried on by nutritionists, biochemists, and animal husbandmen on basic and applied problems related to feeding and management of cattle for beef. The work is in progress at Beltsville, Md.; in cooperation with State experiment stations at federally owned stations in Miles City, Mont.; Fort Robinson, Nebr.; Fort Reno, Okla.; Jeanerette, La.; Brooksville, Fla.; Front Royal, Va.; Newell, S. Dak.; in cooperation with State experiment stations at Tifton, Ga.; and College Station, Tex.

There are contracts totaling \$251,754 with the California, Kentucky, Florida, Wisconsin, and Nebraska Agricultural Experiment Stations. These projects are concerned with the methods of feed preparation and level of grain in the ration, the sites and amount of starch utilization in the ruminant digestive tract, ration components which control feed intake, graded levels of energy intake upon reproductive performance in beef cattle and management of males of beef and dairy breeding for beef production. These projects are for either 3 or 4 years (1964-1967 or 1968), and their basic purpose is to determine the potential for increased utilization of grains for beef production.

There is one grant involving Public Law 480 funds with the Agricultural College in Poznan, Poland. The project is to determine the trace mineral content of forages as affected by stage of growth and methods of harvesting and storing. The project is for a five-year period (1963-1968) and is supported by \$47,311.66 equivalent in Polish zlotys. Another grant with the Institute of Biochemistry and Biophysics in Warsaw, Poland, is to determine the metabolic pathway of protein biosynthesis in the liver microsomes of the guinea pig. The project is for five years (1963-1968) and is supported by \$60,411.46 equivalent in Polish zlotys.

The Federal scientific effort devoted to research in this area totals 12.3 professional man-years. Of this number 4.1 are devoted to digestion and metabolism; 1.5 to concentrates; 1.4 to forage preservation and utilization; 0.7 to nutrient requirements; 3.0 to range and pasture management; 1.1 to management practices; and 0.5 to behavior.

PROGRAM OF STATE EXPERIMENT STATIONS

The States have research in progress on the basic functions of the rumen, particularly the animal-feed interrelations which are responsible for bloat' efficient feed digestion, and the synthesis of essential nutrients. (Additional investigations of rumen function appear in problem area #1.)

The basic requirements of beef cattle for specific nutrients, their metabolism, interactions, and availability in feeds are receiving attention at a number of stations. Some of the topics being investigated are: (1) The requirements, metabolism, and interactions of the many major and trace nutrients; (2) the effect of feed additives or implants upon growth and feed efficiency; (3) the relation of nutrients to metabolic disorders; (4) the toxicity of molybdenum and fluorine; (5) the value of irradiated feeds and assimilation of fallout products; (6) the use of roughage concentrate ratios and chemical regulators for feed intake control; (7) the effect of physical form of the ration upon nutritional value; (8) a reevaluation of the vitamin A requirements and factors affecting them made necessary by apparent deficiencies on rations with ample carotene based on earlier standards.

The efficiency of feed use is being improved through investigations of concentrate and forage feeding. The problems and advantages of all-concentrate or high-concentrate feeding have high priority at this time. The comparative values of various kinds and combinations of feeds and the effect of different physical forms (chopped, pelleted, etc.) are being studied. There is considerable emphasis on development of methods for evaluation of forage crops. Four cooperative regional research committees (NC-64, NE-24, S-45 and W-34) have all or a significant part of their project devoted to this evaluation.

Management problems are being investigated. The main topics are combinations of pasture and drylot feeding; maximum use of pasture throughout the season, especially extending the period by using late fall and early spring pasture crops; supplementation needed when low quality roughages are fed; and creep feeding vs. non-creep feeding of calves.

The State stations have 143 professional man-years devoted to beef cattle nutrition and management.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Digestion and Metabolism

1. <u>Digestion and balance studies</u>. When comparing rations containing 50% cracked corn and 50% sweet potato trimmings, steers consuming the sweet potato ration gained less weight (0.79 vs. 1.03 kg./day) and had lower grading carcasses (low good vs. low choice). Dry matter and crude protein were less digestible in the ration containing the sweet potato trimmings. It may be calculated from these data that sweet potato trimmings contain approximately 80% of the nutritive value of corn. (AH d2-14)

The effects of source of nitrogen and source of carbohydrate on nitrogen utilization were studied using purified diets. Digestibility of nitrogen from urea was greater than nitrogen from isolated soy protein, and starch promoted significantly greater digestibility of nitrogen than did a combination of starch and cerulose. Due to lowered urinary losses, nitrogen retention was greater for the isolated soy protein rations. Feeding the starch ration resulted in greater ruminal molar percentages of acetate and lower percentages of butyric plus higher volatile fatty acids. Information such as this is useful in predicting response differentials by cattle when fed different cereal grains or nitrogen supplements. (AH d2-37)

2. <u>Salivary secretion</u>. Salivary flow was not altered by varying the source of nitrogen (urea vs. soy protein) or carbohydrates (starch vs. cerulose) in purified diets fed to steers. Rate of flow was greater and buffering capacity of the saliva was less during the p.m. than during the a.m. collections. Ruminal a.m. pH was higher and ruminal ingesta weight, volume, and dry matter content were less when steers consumed rations containing urea as the nitrogen source. (AH d2-37)

Daily salivary secretion was 33.5, 45.2, 52.0 and 54.1 liters when steers averaging 350 kg. in weight were fed intakes of .8, 1.4, 2.0, and 2.6% of empty body weight, respectively. Although rate of salivary secretion was higher during intervals when the steers were eating, the response to level of feed intake during eating and resting intervals was essentially the same. (AH d2-37)

Steers were fed 89% or 25% roughage rations as pellets or as coarsely ground mixtures. Resting salivary secretion was greater when feeding the coarsely ground 89% roughage ration (2.0 liters per hour) than when the other rations were fed (1.4 to 1.6 liters per hour).

An adequate flow of saliva is required for proper digestion, and information such as that presented above should lead to feeding and management procedures favoring optimum digestive function. (AH d2-37)

3. <u>Metabolic diseases</u>. In cattle studies at College Station, Texas, in cooperation with the Texas Agricultural Experiment Station when cattle were fed out at various locations in Texas and when cattle and feed were from common origins, location apparently did not play a major role in urinary calculi formation. (AH d2-31)

At the Beeville, Texas, Substation cottonseed hulls, as the source of roughage, produced the greatest number of steers with uroliths and the largest quantity of stones. Approximately 50% of the steers which developed uroliths had received cottonseed hulls in the diet. Steers consuming flax shives as a roughage had the smallest incidence of uroliths followed by ammoniated rice hulls, rice hulls, and an all concentrate ration in that order. (AH d2-31)

Six percent NH₄Cl was added to 29% protein range cubes and fed to 50 weanling calves under normal range management conditions at a private ranch in Nebraska. While consumption was limited to a maximum of 0.75 lb. of cubes daily, the level of NH₄Cl (.07 oz.) was sufficient to decrease the incidence of urinary calculi as compared to a group of 50 calves not receiving the treatment (one case in the treated group as compared to four in the untreated group). (AH d2-31)

In field trials involving 1009 beef cows (S. Dakota) one loss occurred among 505 cows supplemented with magnesium (6 g./day) and calcium (12 g./day), while 18 cases of grass tetany (11 deaths) occurred among the 504 unsupplemented cows. (AH d2-35)

4. <u>Pesticide residues</u>. In a feeding experiment at Tifton, Georgia, corn silage containing 1 to 7 ppm. of dimethoate was fed to beef steers. No effect on blood cholinesterase activity was observed. Feeding silage produced from corn treated with technical grade dimethoate (7% impurities) caused a reduction in feed intake and seemed to impair animal health. This was not true when corn was treated with pure dimethoate. (AH d2-32)

There was no apparent effect from exposure to the pesticides Diaginon and Cygon on ruminal microbial gas production. Preliminary observations do not suggest a differentiating effect of the pesticides on the protozoal population (AH d2-32)

Current cooperative studies with Pesticide Chemical Research Branch on malathion and DDT are concerned with the dissipation of malathion and its metabolites after a massive dose or during low level chronic dosing. Tissues being studies included fat, liver, and blood. Similar studies are being initiated with DDT, except that distribution of the chemical among the fat depots and muscle will also be investigated. (AH d2-32)

A cooperative pesticide study with the Virginia Experiment Station has been initiated at Front Royal, Virginia. Rate of accumulation, techniques, and management procedures which may change the rate of dissipation of the pesticide (heptachlor) from the body tissues of steers are being studied. Mature beef cows are being used to study the effects of heptachlor in the diet (contaminated alfalfa) of pregnant and lactating cattle. Presence of the pesticide will be checked in the cows, the newborn calves, the milk, and nursing calves. (AH d2-43)

5. Microbiology. The effect of implanted diethylstilbestrol (DES) on the rumen microbial population in steers fed 90% corn vs. 90% wheat was examined. Two out of ten steers fed wheat contained ruminal ciliated protozoa (Holotrichs) but none were found in the ruminal fluid of steers fed corn. The number of protozoa did not appear to be related to DES treatment. Total viable counts were higher in steers fed corn and lower in steers fed wheat when DES was implanted. Presumptive identifications of the predominate strains of ruminal bacteria present by treatments are being made. (AH d2-26)

An 'artificial rumen" continuous culture used to study the effect of pH on the ruminal microbial ecosystem indicated that at decreased pH's the bacterial cellulolytic activity is affected to a greater degree than the amylolytic activity, that the bacterial population changes qualitatively with pH changes, and that DNA may be a more reliable parameter to measure microbial population quantitatively than viable counts. (AH d2-26)

When steers were switched from a 75:25, concentrate to roughage, diet to a purified diet containing urea as the only nitrogen source, the following ruminal changes occurred: bacterial counts, molar percent of butyric plus higher acids, total concentration of volatile fatty acids and ammonia concentrations increased while protozoal counts, molar percent of acetic acid, and pH decreased. (AH d2-26)

B. Nutrient Requirements

1. Reproduction. An identical twin Angus cow weighing 930 lb. gave birth to a normal 51 lb. heifer calf, after being raised since weaning on a protein-free diet. A purified diet containing urea as the only nitrogen source was fed during this time. The 1030 lb. co-twin was fed an equal amount of nitrogen and calories in a diet of conventional feedstuffs and gave birth to a 60 lb. heifer.

Currently, bull and heifer calves are being weaned at 82 days of age and placed on purified diets to further study the dietary needs of cattle for growth, reproduction, and lactation.

Different levels of urea in conventional diets are being used to study the long-term effects on the reproductive performance of 12 sets of identical twin beef females. Rations containing none, 1/3 and 2/3 of the total protein equivalent as urea are being compared. After 363 days on test, the gains have been approximately the same for all treatments. The heifers are bred when the lightest twin of a pair reaches 600 lb. (AH d2-22)

The Polish studies on the reactions in liver microsomes during <u>in-vitro</u> protein biosynthesis indicate that Asp-sRNA did not abolish the inhibitory effect of oxaloacetyl-sRNA Asp. (E21-AH-7)

C. Concentrates

1. High concentrate rations for finishing steers. Yearling steers were fed for 98 days rations containing more than 90% corn, wheat, or combinations of these grains. Steers fed all corn or 2/3 corn and 1/3 wheat gained faster than steers fed 2/3 wheat and 1/3 corn or all wheat. Daily gains were 3.1, 2.9, 2.5, and 2.6 lb. for these groups, respectively. Carcass data were similar for all groups. Ruminal samples taken shortly before slaughter from the cattle receiving 2/3 and all wheat were more acid and contained more VFA and NH₃ than samples from steers on 2/3 corn or all corn. Determinations of the plasma amino acids showed that the cattle fed the wheat ration had less aspartic acid, serine, and leucine but more lysine and urea than cattle fed the corn ration. Implantation with 24 mg. stilbestrol resulted in a greater concentration of plasma glycine.

In a 112 day feeding trial, steer gains were depressed when 5% soy oil was added to and 15% urea deleted from a ration of more than 90% cracked corn during the last 56 days of the test. Ruminal ammonia concentrations and rib eye areas were less when the soy oil was added and the urea deleted. (AH d2-37)

At Fort Reno, Oklahoma, a basal ration of barley supplemented with soybean meal, calcium, and vitamin A was fed. Cobalt supplementation tended to increase weight gain, feed consumption, carcass grade, marbling score, and rib eye area. Zinc supplementation alone did not affect growth performance and carcass traits. Combined cobalt-zinc supplementation interacted in such a way on feed/gain and rib eye area as to suggest that zinc may be a limiting trace mineral when the cobalt level is adequate. These results indicate that an "all-barley" type fattening ration is deficient in cobalt and perhaps marginal in zinc.

When feeding rations of about 70% milo to fattening calves, replacement of cottonseed meal with urea decreased gains but improved feed cost per unit of gain. The addition of corn oil to a milo-urea ration did not improve performance, but the addition of a complex vitamin-trace mineral mixture plus corn oil did improve the rate of gain of calves. Trials with identical twin calves fed high milo rations indicated that neither the addition of urea to cottonseed meal nor the replacement of cottonseed meal with soybean meal was of benefit. The replacement of cottonseed meal with fish meal or the addition of copper to a urea containing ration improved rate and efficiency of gain. (AH d2-37)

2. <u>Finishing cattle</u>. When feeding "high" urea rations, based on snapped corn, to beef calves (400 lb.), alfalfa meal appeared to be necessary but was of no advantage when feeding yearling beef steers (700 lb.) on similar rations. The final decision to use urea will depend upon the comparative cost of all feed ingredients. (AH d2-14)

The effects of various levels of roughage in rations for fattening steers were studied in two experiments at Tifton, Georgia. Roughage levels varied from 10 to 50% of the ration. There was no advantage to feeding a small amount of unground hay. Daily gains were not markedly or consistently affected by level of roughage in the ration. In another experiment it was observed that full-fed steers perform better than steers restricted to 92.5 or 85.0% of the intake of steers which were full-fed. (AH d2-14)

Wintering rations ranging from 0 to 100% steam rolled barley were fed to beef calves in studies at Newell, South Dakota. The calves offered all hay were allowed to feed ad libitum, and the barley was substituted for the hay on the basis of its available energy content. Gains during the winter and subsequent summer (pasture) were similar for all groups. The results indicate that beef calves can be wintered satisfactorily by substituting barley for part or all of the hay in wintering rations. The barley was worth about twice as much per 1b. as a 50:50 prairie hay-alfalfa hay mixture.

When rations containing from 50 to 95% barley were fed to finishing steers, daily gains increased somewhat as the amount of grain in the ration increased (2.5 to 2.7 lb./day). There were no differences in carcass grades when the steers were fed to a common slaughter weight.

Restricting feed intake to 85% of full feeding decreased gains and outside fat cover but did not consistently affect carcass grades and rib eye areas. (AH d2-35)

Studies on cereal grain utilization, as affected by method of preparation were conducted at Davis, California. Feed consumption was reduced when grains (corn, milo, and barley) were rolled after 1 1/2 min. of steaming at 60 pounds per square inch (psi). Gains were improved when the grains were rolled after 1 1/2 min. of steaming at 20 psi. Although it was not conclusive, the response appeared greater at the 60% than at the 40 or 80% grain levels. The response in feed efficiency paralleled the response in gains. (AH d2-38)

Studies conducted with steers at Kentucky indicate that the quantity of starch passing into the abomasum increases with increasing levels of starch intake. Thus, post-ruminal starch digestion assumes greater importance in overall starch utilization as dietary starch intake increases. (AH d2-39)

D. Forage Preservation and Utilization

1. <u>Utilization of coastal bermudagrass</u>. The final years' study on six systems of utilizing coastal bermudagrass indicated trends similar to those reported for the earlier trials conducted at Tifton, Georgia. For the three years, the average daily gains for the various systems were: continuous grazing, 1.31 lb.; rotational grazing, 0.89 lb.; strip grazing, 0.86 lb.; feeding pellets, 1.76 lb.; feeding dehydrated hay, 1.48 lb.; and feeding fresh green chop, 0.81 lb. Feed consumption figures are correlated with gains. (AH d2-42)

2. Feeding value of pelleted feeds. See section A, 2.

E. Range and Pasture Management

1. Range supplementation studies. At Brooksville, Florida, cows given injections of 6 million units of vitamin A in November and in April showed no advantage in 205-day weight, calf survival, or pregnancy rate when compared to cows receiving no vitamin A injections. (AH d3-2)

Range management studies at Miles City, Montana, continue to indicate that calf gains during the spring period (4/27 to 6/4) are higher on introduced grass pastures of crested wheatgrass and Russian wild-rye than on native range. In addition, stocking rates (animals/acre) are also greater on the introduced pasture. Percent pregnant at the end of October was excellent for cows pastured during the spring on either the native range (92.3%) or the introduced pastures (100%). (AH d3-1)

At Tifton, Georgia, preliminary studies indicated that steers grazing oats and rye (January through March) outgained (2.1 vs. 1.1 lb./day) steers grazing wheat and barley. Compensatory gains were made in drylot by the steers previously grazed on wheat and barley but estimated feed costs per pound of gain were greater. (AH d2-42)

Steers grazed on oats and then millet averaged 1.8 lb./day gains in studies conducted during three years. (AH d2-42)

Summarizing long-term studies conducted at Fort Reno, Oklahoma, it was noted that adequate nutrition is just as critical in the lactating two-year-old as in the yearling heifer. Earliness of calving and promptness of rebreeding of two-year-old heifers depends largely on the level of nutrition provided during the critical wintering period. (AH d2-36)

Studies of the cumulative influence of level of wintering on the lifetime performance of beef females through five calf crops were conducted. This research indicates that rather than select a level of wintering for the lifetime of the cow, consideration should be given to the life cycle feeding approach in which higher levels are used during growth and development of the female followed by lower levels after the cow has reached maturity. (AH d2-36)

F. Management Practices

1. Management of cattle and pastures for beef production. Winter feeding experiments at South Dakota indicate that when wintering steer calves, there is an advantage in feeding for gains of 1.5 lb./day instead of 1.0 lb./day. This advantage is apparent in total gains, whether the cattle are subsequently fed in drylot or pastures. Steers receiving up to 20 lb. of sorghum silage per day performed as well as steers receiving hay as a source of roughage.

Total winter and summer gains of steers implanted with 12 mg. of stilbestrol in the fall (shortly after weaning) or 24 mg. in the spring compared favorably. There was no advantage to implanting in both fall and spring. There was an advantage to implanting (12 mg.) when steers were subsequently placed in the feedlot. (AH d2-35)

Wintering steer studies at Tifton, Georgia, demonstrated that the feeding value of 5-week-old bahia and coastal bermudagrass hays are similar. The 5-week cuttings of hay support better steer gains than that of either of these hays when cut at 8 weeks of age. (AH d2-42)

The trace element study of soils and forages in Poland continues to show that there are great differences in microelement content of forages depending on species, stage of vegetation and geographic area. (E21-AH-6)

Studies designed to identify and define factors associated with meat quality as affected by maturity and nutritional treatment have been initiated at Beltsville, Md. These studies are being conducted in cooperation with Market Quality Research Division. Beef calves are being (1) full fed, (2) fed to gain about 1 lb./day, or (3) fed to gain about 1 lb./day until six months prior to slaughter and then full fed. Steers within each of these groups will be slaughtered at ages of 6, 12, 18, 24, 30, 36, 48, 60, and 84 months. Physical, chemical, and organoleptic evaluations will be conducted on the carcasses.

G. Behavior

1. Feeding. Reversing 12-hour light:dark intervals also reversed feeding and drinking behavior patterns of growing heifers. Sixty-five percent of the time at the feeder and waterers occurred during the light intervals irrespective of time of day. The extent of the effect varied with animals. The results suggest that light is a potential tool for controlling feeding behavior of cattle.

Preliminary observations suggest that time spent at the feeder daily decreases during estrus.

Feeding patterns of steers consuming all concentrate rations (90% corn) are similar to the feeding patterns of steers consuming roughage containing rations.

Contracted experiments at the Florida Agricultural Experiment Station on the formulation of supplements to control feed intake by beef cattle indicated that the use of salt is limited due to the variability among steers in their tolerance for salt. Animal fat and menhaden fish oil (10%) limited intake of the supplement and showed promise for future studies. (AH d2-40)

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Forage Preservation and Utilization

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PRODUCTION INFLUENCES ON BEEF Animal Husbandry Research Division, ARS

Problem. Beef, lamb, pork, and poultry are excellent sources of wholesome and digestible animal proteins and fatty acids necessary in maintaining a healthy, appetizing diet. However, these meats must be of high quality, as well as in plentiful supply, if they are to retain their high position and esteem in the minds of consumers. Proper finish, a high proportion of lean, with adequate intramuscular fat, tenderness, full flavor, and color desired by the consumer are the goals the meat producer must strive to attain through breeding, feeding, and management. The quality of cuts and kind of meat are directly reflected in the demand and in the price of the product.

Egg shell strength and yolk quality, strength of wool, fatness, quantity, flavor, color, and tenderness of meat are all known to be influenced by production practices. However, these quality characteristics and many more are not well understood, even though they are of considerable economic importance. Effective measures of evaluating quality differences are of great importance in determining the nature and effect of production practices on the products.

USDA AND COOPERATIVE PROGRAM

This is a continuing program conducted by food product technologists, wool and fiber technologists, biochemists, chemists, physiologists, statisticians, and animal husbandmen engaged in both basic and applied research designed to develop methods and information which will be useful in evaluating quality and quantity of animal products and will be useful in aiding and directing livestock production. Research on beef, veal, lamb, and pork is directed at the influence of selection and breeding, nutrition, physiology, management, and other production variables on carcass and meat quality and quantity. Standards are being applied and adapted for appraisal of slaughter animals, of carcasses, and of meat cuts. The objective of the work with poultry and eggs is to ascertain those factors of nutrition, breeding, and management which contribute to the initial quality of poultry products and their capacity to retain that quality. Studies with wool, fur, and fiber are conducted to determine the physical, chemical, and biological structures and properties of wool and other animal fibers as influenced by production factors. The work is conducted at Beltsville, Maryland; Dubois, Idaho; Fort Wingate, New Mexico; Glendale, Arizona; and in cooperation with four State experiment stations. Cooperation is also carried out with the Eastern and Western Utilization Research and Development Divisions, the Human Nutrition Research Division, the Agricultural Engineering Research Division, and the Market Quality Research Division.

PROGRAM OF STATE EXPERIMENT STATIONS

Beef. The influence of feeding and management treatments on carcass and meat characteristics include fattening on grass, drylot, or combinations of these; varying the length of heavy silage feeding preceding finishing with a high-energy ration; creep feeding versus no creep feeding during the nursing period, and various combinations of ration ingredients with and without adjuvants. Many of the projects include economic considerations as well as consumer acceptance and laboratory analysis for quality. Regional project NC-58 is designed to objectively identify the factors that characterize differences in beef carcasses, evaluate the relative importance of these factors, and find the best indicators of these carcass traits in the live animal.

A number of breeding projects contributing to regional research projects NC-1, W-1, and S-10 are designed to determine the effectiveness of selection in improving carcass traits. Other independent studies include the importance of beef conformation as contrasted with dairy type in the production of consumer acceptable beef.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Beef

1. Tenderness evaluation techniques. A study of cooking methods for beef steaks in tenderness studies indicated that variability could be reduced markedly by broiling for 46 minutes at an oven temperature of 175°C, instead of broiling to an internal temperature of 80°C. There were no significant differences in the palatability scores for the two methods. However, there appeared to be better agreement among judges for individual samples, and the judges were better able to reproduce their evaluations on replicate samples. When time constant cooking was used, the observed differences were due almost entirely to sample differences.

A chemical analysis, before and after cooking of selected muscles, judged by the panel to be very tough or very tender, indicated that the acidic groups in meat are responsible for a major portion of the buffering capacity of meat. The buffering capacity of meat between pH 7.0 and 2.5 increases during cooking as would be expected if the protein molecules were being denatured. While the acid buffering capacity for raw and cooked meat differ, the relationships between raw samples parallels that of the cooked samples and could be used to predict the behavior of cooked samples. A moderate correlation exists between tenderness and acid buffering capacity, at least when extremes are studied. (AH d4-5)

The tenderness press was modified by replacing the intermittent pressure increase system with a constant-flow rotary vein pump. Based on tests with 375 beef rib roasts, this modification increased the effectiveness of this instrument in measuring tenderness. Correlations between the panel tenderness score and the tenderness press were r=.13 for the original, and r=.35 for the modified press.

Objective tenderness measures (Warner-Bratzler, STE puncture and shear, and tenderness press) correlated more closely with panel tenderness score for Standard and Utility grades of beef than for Choice and Good grades. Carcass grade and objective tenderness measures, respectively, accounted for 8.1 and 43.8% of the panel score variance among 306 beef rib roasts using the modified tenderness press as one of the four objective measures. (AH d4-4)

2. <u>Composition</u>. Continued studies of estimating carcass yield, selected linear carcass measurements as estimates of wholesale cut yield and composition indicate the measurements are of value. The data showed the cross-sectional shape of the <u>P. major</u> muscle to be that the mathematical considerations of the shape as an ellipse produced a good fit as compared to actual area as determined from a tracing. The results indicated distinct breed differences in area of <u>P. major</u> and total separable lean in 9-11 rib samples. For example, the simple correlation between calculated area of <u>P. major</u> and total lean in the 9-11 rib sample was .91 for Angus, .84 for dual-purpose Shorthorn, .82 for Hereford, .81 for Jerseys and .75 for Holstein. (AH d4-7)

The average computed percentage yield of trimmed, boneless major cuts of 139 beef carcasses and 21 dairy cow carcasses grouped by grade were: Prime, 49.8; Choice, 50.3; Good, 51.3; Standard, 53.1; Utility, 53.9% for the beef-type carcasses; and Commercial, 52.9; Utility, 52.6% for dairy cow carcasses. The computed percentage yields of the cuts of beef-type carcasses were not significantly greater than those of dairy cow carcasses of a similar grade. However, the beef-type carcasses had a significantly higher yield of major cuts in the Standard and Utility than in the Good and Choice grades due to differences in fatness. (AH d4-7)

Ultrasonic measurements of depth of fat and lean over the twelfth rib of finished beef animals measures with acceptable accuracy the depth of fat covering, but not the degree of thickness of muscling. (AH d4-7)

3. Quality in beef muscle

(a) <u>Juiciness</u>. Water-holding capacity is a characteristic of meat tissue that may have a relation to the ability of proteins to "bind" water during aging and cooking. Some meat research workers believe that water-holding capacity is also related to juiciness of the cooked tissue. To test these ideas, 89 beef samples were evaluated for juiciness by panel.

values for centrifugally extracted moisture (CEM) ranged from 15.3 to 32.4% and averaged 24.8%. The CEM values correlated significantly with the panel juiciness score (r=.44) and overall desirability score (r=.32). Thus juiciness and water-holding capacity have a definite and positive relationship. (AH d4-6)

- (b) Electrical properties. Data on electrical properties of approximately 100 beef carcasses have been recorded at three post-mortem intervals. Brass and silver electrodes were used. Preliminary evaluation of the data indicates that, immediately post-mortem, certain electrical properties may be curvilinearly related to specific characteristics of tissue after heating. (AH d4-6)
- (c) <u>Method of preparation</u>. Deep-fat frying vs. oven roasting, has shown a decided advantage in favor of deep-fat frying for bringing out differences in palatability of beef. Differences in flavor, juiciness, tenderness, and overall desirability were noted between breeds and certain lines of cattle when one-inch steak samples were heated in deep-fat. However, when 9-11 rib roasts from these same animals were compared, only tenderness differences were found. (AH d4-4)

PUBLICATIONS -- USDA AND COOPERATIVE RESEARCH

Beef

- Alsmeyer, R. H., Bollinger, N. C., Thornton, J. W., and Hiner, R. L. 1964. Electrical properties and beef palatability. J. Animal Sci. 23(1):858. (Abs.) (AH d4-6)
- Alsmeyer, R. H., Thornton, J. W., Hiner, R. L., and Bollinger, N. C. 1965. Some dorsal-lateral location tenderness differences in the longissimus dorsi muscle of beef and pork. J. Animal Sci. 24(2): 526-530. (AH d4-4)
- Hiner, R. L. 1965. Slaughtering, Cutting, and Processing Beef on the Farm. Farmers' Bul. No. 2209. (AH d4-4)
- Oltjen, R. R., Davis, R. E., and Hiner, R. L. 1965. Factors affecting performance and carcass characteristics of cattle fed all-concentrate rations. J. Animal Sci. 24(1):192-197. (AH d4-7)

INFECTIOUS AND NON-INFECTIOUS DISEASES OF CATTLE Animal Disease and Parasite Research Division, ARS

Problem. Losses from infectious and non-infectious diseases of cattle, other than those due to parasites, are estimated at approximately \$600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA AND COOPERATIVE PROGRAM

The <u>Department</u> has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The <u>Federal</u> scientific effort devoted to research in this area totals 29.0 professional man-years. This effort is divided among sub-headings as follows:

Brucellosis of Cattle 2.5 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the University of Minnesota, the University of Wisconsin, and with the Ohio Agricultural Experiment Station. A project on the immunizing effect of Brucella cell wall is in progress at the Hebrew University, Jerusalem, Israel, under a PL 480 Grant of funds equivalent to \$31,950.00 over a 3-year period.

<u>Vibriosis of Cattle</u> 2.0 at the National Animal Disease Laboratory, Ames, <u>Iowa</u>, and under a cooperative agreement with the New York State Veterinary College at Ithaca.

Tuberculosis of Cattle 2.0 at the National Animal Disease Laboratory, Ames, Iowa, and through a contract with the Michigan State University at East Lansing.

Mucosal-Respiratory Disease-Complex 3.5 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Colorado State University at Fort Collins, the Agricultural Experiment Station, Purdue University at Lafayette, Indiana, and the Iowa State University, Ames.

Mastitis of Cattle 3.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Respiratory Disease of Cattle (Shipping Fever) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Infertility in Cattle, other than Vibriosis and Trichomoniasis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Epizootic Bovine Abortion 0.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California, Davis.

Foot Rot (Infectious Pododermatitis) of Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Etiological, Cytological and Histochemical Studies of Pulmonary Adenomatosis in Cattle 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Immunization against Bovine Leptospirosis 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Chemotherapy in Leptospirosis 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Enteritis of Young Calves 0.5 at the National Animal Disease Laboratory, Ames, Iowa, and under a contract with the University of Idaho, Moscow.

Bovine Lymphosarcoma 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Paratuberculosis of Cattle (Johne's Disease) 2.0 at the National Animal Disease Laboratory, Ames, Iowa

Keratitis (Pink Eye) 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

PROGRAM OF STATE EXPERIMENT STATIONS

The State experiment stations are devoting an increasing amount of effort toward research directed at the prevention, control and eradication of cattle diseases. The objectives of these studies are concerned not only with more efficient production of meat and milk, but also with the production of products which are wholesome and safe for human consumption.

Many of the Western states are cooperating (W-88, Enteric Diseases of Neonatal Calves) in initiating basic and applied studies to determine the cause and control of intestinal infections in calves.

Increasing interest and support is being given to the cause and control of bovine leukosis. Workers also seek basic information concerning the possible relationship of this disease to cancer in man and other animals.

Cooperative regional studies, among the Southern (S-30, Diseases of Reproduction) and Northeastern states (NE-40, Pathology of Breeding Failure), seek to determine the relation of various infectious agents to poor reproductive performance and sterility in cattle. Increasing attention is being directed toward the role of viruses in infertility. A new vaccine against vibriosis offers promise. However, antigenic variation in strains of the organism causes continued concern to workers. The role of leptospira in infertility is being determined. Basic studies pertaining to the diagnosis and pathogenicity of the different serotypes of the organism continues to receive considerable attention.

The North Central states are cooperating (NCR-29, Shipping Fever of Cattle; NCR-37, Mucosal Disease of Cattle) to determine the inter-relationships between various agents and factors associated with respiratory infections in cattle. The relation of virus diarrhea and infectious bovine rhinotracheitis to the respiratory disease complex is being given considerable attention. Preventive vaccines are being developed and evaluated under laboratory and field conditions.

Many of the states, particularly those in the north central region, (NCR-47, Mastitis in Cattle) are cooperating informally in seeking to determine the cause and effective methods of controlling mastitis in cattle. Preventive and therapeutic agents are being evaluated to determine their efficacy. Residue studies are an important part of these investigations.

Attempts are being made to determine the role of certain viral agents in foot rot and infectious keratitis (pink eye).

Much effort is being made to develop means of controlling urinary calculi in cattle (Regional Research project, W-41, Urinary Calculi of Cattle). Consideration is being given to the theory that an imbalance of certain nutritional elements may contribute to the development of the condition.

Sporadic diseases and new problems not previously encountered often become economically important enough to require intensive investigation. Other bovine disease conditions currently under investigation include epizootic bovine abortion, toxicoses, ketosis, parturient paresis, white muscle disease, aplastic anemia, enterotoxemia, porphyria, tuberculosis, paratuberculosis, various abnormalities, bloat, brucellosis, Q fever, etc.

Increased attention is being paid to public health aspects of animal disease research. Greater emphasis is being placed on research which would control and eradicate animal diseases transmissible to man. The protection of the consumer against foodborne diseases is also receiving considerable support.

The total State scientific effort devoted to diseases of cattle is 132.1 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Brucellosis of Cattle

In a research study conducted at the National Animal Disease Laboratory (NADL), Ames, Iowa, fifteen bulls from 4 to 10 months old were vaccinated against brucellosis to determine the nature and frequency of persistence of Brucella abortus Strain 19. A mild transient postvaccinal orchitis was detected in 8 bulls. Postvaccinal brucellemia was detected in 13 of the 15 bulls and persisted up to 28 days in one bull. Semen samples collected from the bulls had no noticeable decrease in quality and did not contain Brucella. Postvaccinal serum agglutinin titers persisted longer at diagnostic levels in bulls than in heifers vaccinated at the same age in a previous experiment. Ten of the 15 bulls still had "reactor" or "suspect" titers at 18 months of age.

The bulls were killed at 18 months of age and at necropsy there was no evidence of gross or microscopic pathologic alterations attributable to Strain 19, nor could the organism be demonstrated in any of the tissues. Under the conditions of this experiment Strain 19 did not localize or persist in any of the 15 bulls. However, persistence of serum agglutinin titers in bulls of breeding age may confuse interpretation of test results and hinder the progress of brucellosis eradication. (NADL) (ADP al-3(R)

The University of Minnesota, under a cooperative agreement with the USDA, reported finding that the number of animals in the herd influences the efficiency and sensitivity of the Brucella Ring Test (BRT). Work is being continued on the development of improved methods for conducting the BRT to increase or decrease the sensitivity according to the number of animals in the herd. The results will greatly increase the efficiency and applicability of the BRT as a screening test for Brucella agglutinins in milk and increase the usefulness of the test in accordance with the increasing size of herds to meet economic and technologic change. (Minnesota) (ADP al-3(R)

Ohio Agricultural Experiment Station, Wooster, under a cooperative agreement with the USDA, reported progress has been made on the first and second phases of the program on serological tests and vaccination of heifer calves at 2 and 3 months of age respectively, with <u>Brucella abortus</u>, Strain 19 vaccine. Some of the heifers 15 to 18 months of age have been bred.

(Ohio) (ADP al-3(R)

The University of Wisconsin, Madison, under a cooperative agreement with the USDA, reported finding a standardized complement fixation test, used in the diagnosis of bovine brucellosis on over 60,000 serum samples during the past three years, has been the most effective of the supplementary serological tests for the detection of infected animals regardless of the tube agglutination test titer. In no case was B. abortus isolated from an animal with a CF titer of less than + at 1:20. Over 90% of proved infected animals had CF titers of + at 1:40 or higher.

Cultures indistinguishable from Strain 19 in CO₂ independence, sensitivity to thionin blue, safranin O erythritol and penicillin have been isolated from 27 cows in Wisconsin, one from Virginia, one from Washington, one from Israel, and seven from New York over the past three years. Ten of these have also been found indistinguishable from Strain 19 in virulence for guinea pigs. These are interpreted as being authentic cases of persistent infection with Strain 19.

A method of dissociating antibody from brucella antigen in tissue by treatment with SM urea has been developed. This has been adapted to improvement of fluorescent antibody staining of <u>Brucella abortus</u> in tissue and to the proving of specificity of staining of antigen in a single preparation. The method should have general application in the fluorescent antibody technique.

(Wisconsin) (ADP a1-3(R)

B. <u>Vibriosis</u>

The New York Veterinary College, Cornell University at Ithaca, under a cooperative agreement with the USDA, reported the following:

Diagnosis of Vibriosis in the Bull by use of the Fluorescent Antibody Technique. A fluorescent antibody conjugate was prepared by labelling the gamma globulin fraction from a pool of rabbit antisera for one strain of Vibrio fetus venerealis with fluorescein isothiocyanate. Nonspecific fluorescence in stained specimens was minimized by using a fraction of the conjugate separate by ion-exchange chromotography. Cross reactions in the fluorescent antibody reaction were observed with intestinal and venereal strains of V. fetus but not with V. bubulus or 17 other species of bacteria tested.

The conjugate was used to stain smears of preputial fluid from a group of 24 bulls. This group included known carriers and bulls of varying ages from which <u>Vibrio fetus</u> had not been isolated. Samples from each bull were examined weekly for 6 consecutive weeks. Complete agreement was obtained between the results of the fluorescent antibody tests on preputial fluid and the results of cultural examination of semen samples from these bulls. The results of this experiment indicate that the fluorescent antibody reaction provides a highly accurate and sensitive method for the detection of <u>V. fetus</u> carrier bulls.

Diagnosis of Vibriosis in the Bull by Isolation of the Organism from Semen. A six-months' study has been completed for the purpose of evaluating different cultural procedures on semen for diagnosing vibriosis and obtaining an estimate of the efficiency of semen culture and as a method for detecting V. fetus carrier bulls.

Five semen samples from each of 35 <u>Vibrio fetus</u> carrier bulls were cultured at monthly intervals. <u>V. fetus</u> was identified in 151 of the 175 samples (86.3%). Of 146 isolates fully characterized biochemically all were classified as <u>Vibrio fetus venerealis</u>. Isolations were made in 5 of 5 attempts from 17 bulls, 4 of 5 attempts from 12 bulls, and 3 of 5 attempts from 6 bulls. On the basis of these results it would be necessary to culture two or three semen samples in order to establish a bull's carrier status with confidence. The sensitivity of this method probably falls somewhat below that of fluorescent antibody tests on preputial scrapings. Direct culturing on antibiotic medium, in which spread plates were prepared from undiluted semen from three serial tenfold dilutions, was found to be a much more efficient method of isolating <u>Vibrio fetus</u> than was culturing of filtered samples.

(New York) (ADP al-9(R)

C. Tuberculosis

Research studies were continued at the National Animal Disease Laboratory, Ames, Iowa. The studies pertained to:

Glycoprotein Levels in Cattle Naturally and Experimentally Infected with Mycobacterium bovis. The study was to determine if serum glycoprotein changes which are used in the diagnosis of tuberculosis in other species might be applicable to the diagnosis of tuberculosis in cattle. In experimentally infected cattle there was a significant rise in the serum glycoprotein during the first five weeks after infection. After this the glycoprotein levels tended to decrease. No evidence was found correlating an increased serum glycoprotein level with either positive skin tests or the presence of lesions in naturally infected cattle. The test does not appear to be of value in the diagnosis of tuberculosis in cattle.

Concentration Effects in Cervical Tuberculin Tests of Cattle Naturally Infected with Mycobacterium paratuberculosis. In determining the effect of simultaneous intradermal cervical injections of two concentrations of ARS tuberculin on the intradermal reactions to each concentration, it was found that simultaneous injections at two concentrations did not significantly affect the size of reactions.

In determining if cattle react differently to the two concentrations, the reactions to full strength tuberculin were significantly larger than those to tuberculin diluted 1:10 whether read at 24, 48, 72, or 96 hours.

In comparing the accuracy of two methods of measuring the reactions, the interpretation of the reactions was the same whether measured by palpating or with a dermal thickness gauge.

An intravenous tuberculin test was used in 76 cattle to supplement but not replace the intradermal tuberculin test in detecting infected cattle which fail to react to the intradermal test. Forty-seven cattle reacted to the intradermal test of which 16 also showed substantial temperature increases with the intravenous test. Fourteen of the 16 had lesions of tuberculosis and 16 that did not show temperature increases also had lesions.

Twenty-nine cattle did not react to the intradermal test, 24 of these did not show temperature increases to the tuberculin administered intravenously. The remaining 5 did show temperature increases, and all 5 were found to have lesions of tuberculosis on postmortem examination.

(NADL) (ADP al-13(R)

Research was continued at the Michigan State University, East Lansing, under two contracts with the USDA. Reports submitted are as follows:

Contract No. 12-14-100-6852(45). The final report on work under this contract was received during the fiscal year 1965. Findings were:-

Polysaccharide specific antibodies and phosphatide specific antibodies, as determined by the hemagglutination (HA) test and the kaolin-phosphatide (KP) test, were elicited in calves by experimental infections with Mycobacterium bovis and M. avium. At necropsy, the calves inoculated with M. bovis had lesions and progressive disease. The calf inoculated with M. avium had lesions and non-progressive disease.

Calves inoculated with pseudochrome and Group IV atypical mycobacteria did not have increased HA and KP serum titers. At necropsy, no lesions or disease were detected.

Calves inoculated with Group III atypical mycobacteria (bovine and porcine origin) had varying HA and KP serum titers. Group III organisms (bovine origin) had a range of virulence from none to that almost equal to M. bovis and those of porcine origin produced few or no lesions in calves. The HA and KP serum titers could not be consistently correlated with disease.

The cows from a gross-lesion herd had fourfold or greater increases in HA and KP serum titers after administration of tuberculin. Two cows from a no-gross-lesion herd did not exhibit the anamnestic-like response. At necropsy the former cows had lesions and progressive disease - the latter cows did not have lesions or disease.

Swine which were inoculated with \underline{M} . \underline{bovis} , \underline{M} . \underline{avium} , and Group III organisms (porcine origin) had lesions at necropsy and fourfold increases in HA and KP serum titers after administration of tuberculin.

Three of four calves, which were inoculated intradermally with heat-killed organisms, had fourfold increases in HA serum titers. None of the four calves had lesions or disease.

The route by which some of the Group III organisms (bovine origin) were administered to cattle altered the serologic response. When inoculated intradermally, all calves had a fourfold or greater increase after the first tuberculin test.

When the three strains of Group III organisms were introduced into the uterus, four of the nine heifers did not have a fourfold increase after the first tuberculin test.

When the three strains of Group III mycobacteria were administered in an aerosol, the HA serum titers were considerably lower.

Specific phosphatide extracts prepared from a representative group of known and atypical mycobacteria did not increase the specificity of the kaolin-phosphatide test.

Generally, swine and cattle with lesions exhibited the anamnestic-like response to the first tuberculin test.

Contract No. 12-14-100-7164(45). In Section I, the study relative to virulence of 12 cultures of microbacteria in calves, swine, guinea pigs, rabbits, and chickens, is approximately two-thirds completed. In Section II the study and comparison of the disease in adult cattle and calves by Group III microbacteria, is nearing completion. In Section III progress is being made in the studies designed to produce and evaluate sensitins for the detection and differential diagnosis of microbacterial infections in laboratory animals. In Section IVa. guinea pigs were inoculated intraperitoneally with M. bovis organisms. Serums were collected from them at intervals of 7 - 10 days post-inoculation. Ouchterlony immunodiffusion was not a satisfactory way to compare serums from normal and tuberculous guinea pigs. A progressive depletion of the alpha globulin lipoproteins and a simultaneous increase in the slow moving alpha globulin lipoproteins occurred in the serums from the tuberculous guinea pigs.

Hyperalpha_-globulinemia was detected by cellulose acetate electrophoresis in the serums from tuberculous guinea pigs. Coincident was the detection by immuno-electrophoresis of an additional alpha_ globulin, tentatively named alpha_-T. Cellulose acetate electrophoresis and immuno-electrophoresis of serums obtained from guinea pigs sensitized to tuberculin with heat-killed M. bovis revealed neither hyperalphaglobulinemia nor the alpha_-T.

Gel infiltration of normal serum in Sephadex G-100 and G-200 was not a satisfactory procedure for the separation of the serum antigens.

Column chromatography on DEAE-cellulose separated normal serum into four major and several minor fractions.

Normal serum was separated into three main fractions by electrophoresis in insoluble potato starch. Satisfactory separation of the serum antigens was not obtained by either of these procedures. Normal serum was separated into five fractions by electrophoresis in agar gel. From seven to nine fractions were readily resolved by electrophoresis on cellulose acetate membranes. Thirty antigens were found in normal serum by immuno-electrophoresis.

Interpretation of the different phases of the work under this contract will be submitted in the final report. (Michigan) (ADP al-13(R)

D. Mucosal-Respiratory Disease-Complex of Cattle

Research work was continued at the National Animal Disease Laboratory, Ames, Iowa. The report shows:

Studies on the intracellular synthesis, separation and characteristics of the soluble antigen of bovine viral diarrhea virus have been completed. Complement fixing soluble antigen was detectable intracellulary before the appearance of infectious virus during synthesis in roller flask cultures of bovine embryonic kidney cells. The release of infective virus into the extracellular fluid was concomitant with the release of soluble antigen.

Soluble antigen was separated from the infective virus. It was heat labile at 56 C, but stable in buffers at pH 5.0, 7.0, and 9.0 at 37C. It was irreversibly precipitated in buffers at pH 3.0 or below. Trypsin and a chymotrypsin completely inactivated the soluble antigen whereas ribonuclease and deoxyribonuclease had no detectable effect on the complement fixing activity. There was no apparent serologic relationship between the soluble antigen of bovine viral diarrhea virus and arbovirus group B and lymphocytic choriomeningitis virus antisera.

A strain of bovine viral diarrhea virus, NADL-MD, was adapted to primary and a cell line of swine kidney cell cultures.

Prior infection of the swine kidney cell line with modified hog cholera virus completely abolished the cytopathic effect and suppressed the yield of adapted NADL-MD virus. The interference occurred prior to the formation of soluble antigen since this was decreased two to fourfold and virus yield was decreased by 90 percent.

Application of the interference test made it possible to study the rate of development of neutralizing antibody against hog cholera virus.

A modified strain of hog cholera virus used to infect swine kidney cell cultures, interfered with the cytopathic effect and virus yield of adapted NADL-MD virus used for challenge. Interference by modified hog cholera virus was dosage dependent and required infection of the cells before interference was expressed. It was demonstrated that interferon prepared

by conventional methods played no role in interference. A swine-passaged strain of hog cholera virus did not produce as complete interference as the strain passaged in swine kidney cell cultures.

When cell cultures were experimentally infected with bovine virus diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) viruses, the latter outgrew the former in relatively few passages, thus indicating that isolation of a mixed population of BVD and IBR viruses from an infected animal could possibly give rise to a pure culture of the latter virus in a relatively short time after the primary isolation.

Calves born to dams devoid of neutralizing antibody against bovine viral diarrhea virus (BVDV), although housed with other mature animals having antibody titers, have not become infected with the agent during a period of 8 months. When dams possessed neutralizing antibody against BVDV, it was transmitted to the calves at the same or higher titers but then decreased over the following 5 months until the serums became negative. In both of these examples the calves were kept with their dams and allowed to nurse. Results to date indicate that BVDV is not readily transmitted in the absence of clinical cases or other as yet unknown factors.

(NADL) (ADP al=14(C)(R)

Colorado State University, Fort Collins, under a cooperative agreement with the USDA, reported that the longevity of immunity of cattle to infectious bovine rhinotracheitis (IBR) has been found to be longer than 5½ years. The quantitative relationship between neutralizing titer and the susceptibility of the cattle will give a guide for vaccination and diagnostic purposes. IBR will produce abortion. (Colorado) ADP al-14(C)(R)

Research investigations were continued at Purdue University, Lafayette, Indiana, under a cooperative agreement with the USDA. The report shows that the apparent incidence of the mucosal disease complex has not changed. Most cases tend to be typical of chronic cases of the syndrome earlier called "virus diarrhea." However, some typical acute outbreaks of "mucosal disease-Iowa" are encountered in the Lafayette area in which mortality approaches 100% of affected animals, and morbidity 15 to 20 percent.

The properties of two cytopathogenic strains of bovine viral diarrhea (BVD) virus, Indiana 1061, and reference strain Oregon C24V, were studied using tissue culture methods. In vitro virus assays were performed in bovine kidney (BK) cell cultures. Indiana 1061 strain virus was isolated from the spleen of a calf in which pathological lesions of BVD were found at necropsy. A mild disease syndrome, indistinguishable from experimental BVD, was observed in calves that were inoculated with spleen suspension. Reciprocal cross-immunity tests demonstrated that calves immunized against Indiana 46 strain BVD virus were resistant to challenge with the new isolant. Calves that had been inoculated with spleen suspension did not react to a challenge injection of Indiana 46 strain virus.

Serum neutralization tests indicated that the newly isolated virus, designated Indiana 1061, was antigenically related to reference strain Oregon C24V. Serums obtained from calves following inoculation with Indiana 1061 and Oregon C24V viruses neutralized the cytopathic effects of Indiana 1061 virus in BK cell cultures.

Bovine kidney cell cultures infected with Indiana 1061 or Oregon C24V viruses, did not absorb guinea pig, bovine or ovine erythrocytes when incubated at 25 C. or 4 C. Erythrocytes added to the culture medium were not agglutinated.

Morphologic changes occurring in BK cells following inoculation with Oregon C24V virus showed the initial degenerative changes were confined to the cytoplasm of the cells and were characterized by condensation and vacuolization of the cytoplasm, followed by obvious nuclear changes. Cavitations in the nucleoli occurred early in the course of degeneration.

Cytochemical staining with acridine orange revealed an increase in cytoplasmic and nucleolar fluorescence (ribonucleic acid) (RNA) at 24 hours. As progressive infection of the cell sheet occurred, cytoplasmic (RNA) fluorescence increased. Treatment of cultures with RNAase completely removed the cytoplasmic and nucleolar staining.

Anti-Oregon C24V fluorescein labeled globulin specifically stained cultures infected with Indiana 1061 or Oregon C24V viruses. Specific fluorescent staining was detectable 16 hours after inoculation and prior to the development of cytopathic changes. Viral antigen was found diffusely spread throughout the cytoplasm, and in some cells was concentrated in a perinuclear location. "Rounded-up" cells showed brilliant fluorescent staining. Large particles or chunks of fluorescing material could frequently be seen in the cytoplasm of degenerating cells. In vacuolated cells, the viral antigen was concentrated at the periphery of the vacuoles. Viral antigen was not observed in the nucleus of cells. Fluorescent staining was completely or markedly inhibited when homologous immune globulin was mixed with conjugates prior to staining.

The Specific-Pathogen-Free (S.P.F.) cattle herd continues to be relatively free of important pathogens with the exception of serological evidence of parainfluenza (SF-4). Titers were highest in animals under one year but colostrum-deprived calves had no titers. The reproductive efficiency of the herd is normal and about 20 calves will be available for research during the next 12 months. (ADP al-14(C)(R)

Iowa State University, Ames, under a cooperative agreement with the USDA, reported the direct fluorescent antibody (FA) test is well suited to the diagnosis of cases of infectious bovine rhinotracheitis (IBR). The method appears to be more trustworthy than isolation.

Immunological tolerance studies with bovine virus diarrhea (BVD) infected calf fetuses have been initiated with the injection of bovine fetuses in the first and second trimester of fetal life. The injection of virulent virus apparently does not cause abortion, or elicit an antibody response in the dam. Calves will be recovered by caesarean section and raised in isolation for three months prior to challenge.

Four strains of IBR are being studied in an attempt to associate basic properties with observed differences in pathogenicity. Although these strains will cross neutralize in standard neutralization tests, kinetic neutralization studies show some differences between strains.

(Iowa) (ADP al-14(C)(R)

E. Mastitis of Cattle

The research studies at the National Animal Disease Laboratory, Ames, Iowa, related to the following:

The nonhemolytic and weakly hemolytic coagulase-negative staphylococci, known as Staphylococcus epidermidis, have been considered nonpathogenic and frequently neglected as a cause of bovine mastitis. The organisms are not typable by bacteriophage and do not produce toxins. Methods for characterizing and differentiating these organisms are needed for enzootiological studies. A study was made to determine whether the various degrees of pigmentation might be useful in differentiating strains. Spectrophotometric analysis of the pigments of 70 isolates, representing 48 strains of Staphylococcus epidermidis, exhibited absorption curves that were classified into seven types, designated I, II, III, IV, V. VI, and a S. aureus type. Two subtypes were included in types I and III. All nonpigmented cell extracts were classified as type I. Three isolates gave an absorption curve that was similar to the curve produced by extracts of five of seven S. aureus strains, thus the designation S. aureus type. The differences in pigment complexes indicated by the various absorption curves of methanol extracts were substantiated by column-chromatography studies. Generally, pigments of types II to VI, as produced by representative strains, were of a xanthophyllic nature, i.e., more soluble in methanol. S. aureus type pigment studied was carotenelike, i.e., more soluble in petroleum ether. Analyses of representative strains showed that the type of spectral absorption curves did not change whether the organisms were carried in vitro and tested through 3 months, or isolated repeatedly from infected udders for periods up to 8 months. The method of determining the spectral absorption curves of whole-cell methanol extracts provides an additional tool for differentiating strains of S. epidermidis that can be used in enzootiological studies of udder infections.

(NADL) (ADP al-15)

The University of California, Davis, under a cooperative agreement with the USDA, submitted a report referring to previous studies that indicated the important role of preleukocytosis (inflammatory response) in protection of the lactating bovine mammary gland against peracute coliform mastitis. Acute mastitis develops when the inflammatory reaction (leukocytes specifically) destroy the flora releasing the endotoxin. High molecular weight polysaccharides of bacterial origin have been tried as a method to prevent diapedesis of leukocytes. Commercially prepared dextran (PHARMACIA Chemicals, Uppsala, Sweden), in increasing amounts and of higher molecular weight than reported as an effective agent, gave no difference in either the speed or magnitude of the leukocytic infiltration in experimentally infected mammary glands than in glands of control cows receiving no dextran. Bacterial levans of higher molecular weight were found to be more effective than dextran in laboratory animals.

Aerobacter levanicum levan was prepared and injected into a cow prior to inoculation of a gland with Aerobacter aerogenes. The injection proved to be fatal to the cow presumably due to an overdose of the levan even though on a weight basis; less levan was injected than was the dextran. The effect resembled that of massive histamine release. No leukocytosis into the inoculated quarter took place during the 8 hours of survival post levan while the control cow developed the typical local reaction within 4 hours.

Several of the cows are now in their fifth lactation. No mammary gland infections with pathogenic staphylococci have appeared in this herd. This is evidence that staphylococcal mammary infections are not the inevitable consequence of aging and wear and tear of the milking act. The majority of these cows, although having experienced many episodes of acute or chronic experimental mastitis, secrete essentially normal, cell-free milk again within a short period of time after the pathogenic bacteria disappear.

Several natural, chronic infections with intermediate coliform organisms and paracolon-like organisms have occurred in some quarters of these cows. These infections have been persistent and have stimulated significant leukocytic infiltrations into the milk (±5.0 millions of cells/ml). Advantage has been taken of this to attempt to superimpose Streptococcus agalactiae or Pseudomonas aeruginosa in repeated daily doses without success. The pre-leukocytosis of ±5.0 million cells prevented the inoculated organisms from multiplying and thus infection was precluded. Daily inoculation of Streptococcus agalactiae or Pseudomonas aeruginosa into glands having undulating levels of leukocytic infiltration have led to infection becoming established when infiltrating leukocyte level became reduced temporarily.

Pseudomonas aeruginosa appeared to be able to establish itself when multiple doses were given in the presence of infiltrating cells at ±1,000,000/ml. of foremilk. Str. agalactiae even in doses of several thousand was held in check by a pre-existing inflammatory response characterized by foremilk cell counts of 500,000. Commonly, cell numbers/ml. in strippings milk are

several fold greater than in foremilk. Strippings counts of several million in the face of foremilk cell numbers of less than 1,000,000 appear to reflect a considerable barrier to both <u>Str. agalactiae</u> and coliform bacteria.

Cultures of fresh milk fail to demonstrate the pseudomonads. Pre-incubation of milk is required for colony growth on culture from chronically infected lactating glands. The <u>Pseudomonas aeruginosa</u> organisms readily grow on culture of fresh fluids drawn from chronically infected dry glands.

Studies are demonstrating the effectiveness of pre-existing leukocytosis in preventing establishment of <u>Str. agalactiae</u> and <u>Ps. aeruginosa</u> when entering the gland in small numbers as a single incident.

(California) (ADP al-15)

F. Respiratory Diseases of Cattle (Shipping Fever)

Research investigations conducted at the National Animal Disease Laboratory, Ames, Iowa, are being continued on basic studies on the physiology of organisms associated with shipping fever. A semi-defined medium for the growth of Pasteurella haemolytica was developed. It consisted of acid-hydrolyzed casein, supplementary cysteine, inorganic salts, and either D-galactose or sucrose as the carbon source. Essential vitamins were pantothenic acid, nicotinamide, and thiamine. The phosphorylated forms of thiamine were more efficient than thiamine itself in promoting growth. Six strains of P. haemolytica, isolated from cases of bovine respiratory disease, grew well in the medium. The medium is being used in studies on the effect of bovine tissue exudates and fluids upon the nutrition and metabolism of Pasteurella species.

Exposure studies in specific pathogen free calves using parainfluenza-3 virus and Pasteurella haemolytica, singly or in combination, produced a clinical syndrome closely resembling "shipping fever."

(NADL) (ADP al-17)

G. Etiology of Infertility in Cattle other than by Vibriosis and Trichomoniasis

In research studies at the National Animal Disease Laboratory, Ames, Iowa, Mycoplasma was isolated, for the first time, from an aborted bovine fetus and from vaginal mucus of cattle with signs of infertility. Morphological and biochemical comparisons were made between the fetal and vaginal strains and 4 strains from other sources. The fetal isolant differed from others in colonial morphology, methylene blue reduction and carbohydrate fermentation. Vaginal isolants were similar in colonial morphology and biochemical properties with the exception of serum requirement. Similarities were also noted between the bovine and non-bovine strains.

Additional work is needed to determine the serological relationship of the fetal and vaginal isolates as well as the importance of Mycoplasma as a cause of bovine infertility.

(NADL)

(ADP al-19)

H. Epizootic Bovine Abortion

The University of California, Davis, under a cooperative agreement with the USDA, reported that previous studies indicated the failure of vaccination to prevent epizootic bovine abortion (EBA) might be because the cattle were vaccinated too near the time of their exposure to the causative agent to allow for the development of a protective immunity. Multiple injections of vaccine, beginning in calfhood and continuing to or following conception, is the present program.

A viable and an inactivated vaccine are being tested in the study. The antibody response has been exceptionally good, but the immunity in these cattle has not been challenged with virulent virus as yet.

Indications are that the EBA virus is becoming attenuated by continued serial passage in chicken embryos and mice.

Preliminary findings indicate that antibiotic therapy might be of some value in controlling EBA when treatment is initiated just before or following exposure to the virus.

It was shown conclusively that the EBA agent is not spread by venereal means as once believed. (California) (ADP al-21)

I. Immunization Against Bovine Leptospirosis

Work at the National Animal Disease Laboratory, Ames, Iowa, shows that bovine leptospira were grown in chemically characterized medium free of serum or serum protein, but the leptospiral growth was poor and the organisms lacked certain surface antigens which are believed to be involved in immunity. The addition of rabbit serum or bovine albumin to cultures of L. pomona in synthetic medium did not restore their antigenicity. It was restored after a minimum of seven generations in medium supplemented with rabbit serum. Various synthetic peptides or fragments of albumin did not replace albumin. The end groups of the albumin molecule were not essential for its function. (NADL) (ADP al-25)

J. Chemotherapy in Leptospirosis

This work is being conducted at the National Animal Disease Laboratory, Ames, Iowa. The minimal, growth-inhibitory concentrations (µg./ml.) of antimicrobial agents and dyes for Leptospira pomona, Leptospira canicola, Leptospira autumnalis, and Leptospira grippotyphosa in synthetic medium were: penicillin G, 0.06; oxytetracycline and chlortetracycline, 0.5; erythromycin, 0.025; tylosin, 0.05; actinoscectacin and kanamycin, 0.25;

dihydrostreptomycin, 0.3; chelocardin and lincomycin, 2.0; fucidin, capreomycin, isoniazide, and chloramphenicol, 20.0; sulfachlorpyridazine, sulfamethoxazone, and sulfadimethoxane, 1000; crystal violet, methylene blue, and pyronin B, 0.2; and thionin and basic fuchsin, 20. Semi-synthetic penicillins (oxacillin, methicillin, and phenathicillin) were five to fifty times less effective than penicillin G. Tylosin, kanamycin, tetracyclines, and penicillins were two to five times less effective in medium supplemented with rabbit serum than in synthetic medium. No differences in sensitivity to dihydrostreptomycin were found among 12 leptospiral serotypes including virulent strains and nutritionally fastidious strains. Dihydrostreptomycin, chlortetracycline, and penicillin at high concentrations affected leptospiral respiration, motility, and viability differently. Dihydrostreptomycin had little effect on the respiration or motility of L. pomona, but subcultures failed to grow; chlortetracycline, but not penicillin, rapidly inhibited all three parameters.

Chemotherapeutic agents, which inhibited leptospiral growth in vitro, were administered to hamsters in attempts to eradicate renal \underline{L} . \underline{pomona} . \underline{L} . \underline{pomona} was cultured from only one of 50 infected hamsters treated with dihydrostreptomycin (25 mg/kg once daily for 3 days).

The following drugs (mg./kg. of body weight once daily for 3 days) were not effective: crystal violet, methylene blue, pyronin B (25); penicillin G (30 and 60); phenathicillin, oxacillin, and methacillin (25); fucidin, kanamycin, nalidixic acid, and capreomycin (25); lincomycin, chelocardin, chlortetracycline, oxytetracycline, tetracycline, chloramphenicol, actinospectacin, tylosin, and erythromycin (12.5, 25, and 50).

Neither chlortetracycline in the feed (1,000 Gm./ton for 10 days) nor dihydrostreptomycin in the drinking water (25 mg./kg. for 7 days) eliminated renal leptospires. (NADL) (ADP al-26)

K. Investigations on Bovine Lymphosarcoma

The report from the National Animal Disease Laboratory, Ames, Iowa, shows that in the investigation of field cases of bovine lymphosarcoma, a blood protozoan parasite tentatively identified as Trypanosoma theileri, has been isolated from the leukocytes in all cases. The parasites can be detected in leukocyte cultures from 1 to 6 days after the cells have been planted. It appears essential that fetal calf serum should be used in the medium rather than serum from the affected animal in order to isolate the parasites consistently. Serum from the affected animal causes the parasite to disappear from the cultures and this may be due to antibodies. Relationship of the parasite to the cause of bovine lymphosarcoma has not been determined. (NADL) (ADP al-30)

L. Paratuberculosis (Johne's Disease) of Cattle

Reports from the National Animal Disease Laboratory, Ames, Iowa, summarizing the work of several years, show there is no known satisfactory method of diagnosing Johne's disease in carrier animals that are not showing clinical signs of the disease.

A herd of cattle, affected by the disease, consisting of approximately 175 animals, was studied for 6 years. Blood samples were obtained from all cattle twice a year and selected tissues of all cattle slaughtered were examined. Of 159 cattle removed from the herd and slaughtered during the study, 111 had high hemagglutination test titers (1:32 or higher). At slaughter the bacillus was harbored by 45 of these of which 22 were showing clinical signs of the disease. Sixteen of the remaining 48 with low hemagglutination titers (1:16 or less), also harbored the bacillus at slaughter and 4 of these had clinical signs of disease. From these results it appears that high hemagglutination titers do not seem to be closely enough associated with active disease to use this test for diagnostic purposes on individual cattle.

Ninety-eight adult cattle from herds infected with Johne's disease were tested by injecting johnin into the jugular vein. Rectal temperatures were recorded just before the injection and at set intervals after injection. A temperature rise of 1.5° F or more over the preinjection temperature, provided the highest temperature exceeded 103.2°F, was considered a significant reaction.

All cattle were slaughtered and examined culturally and microscopically for Johne's disease. On the basis of postmortem findings, they were classified as negative, lightly infected or heavily infected. Eighty percent of the heavily infected and 35 percent of the lightly infected cattle reacted significantly to the test. The remainder did not. The intravenous johnin test appears to have value as a diagnostic test as it has better correlation with postmortem findings than any other test now in use.

(NADL) (ADP al-35)

M. Infectious Keratitis (Pink-eye) of Cattle

At the National Animal Disease Laboratory, Ames, Iowa, in a series of preliminary experiments on bovine pink-eye, workers observed that a mercury sunlamp enhanced the effect of Moraxella bovis infection upon the bovine eye. The resulting disease was indistinguishable from field cases of infectious bovine keratoconjunctivitis (pink-eye). This method made possible the study of the disease under controlled conditions at any time of year. The workers proposed that ultraviolet has a primary etiological role in the disease. Work is being continued. Studies on the etiology of bovine pink-eye have been completed on the isolation and characterization of Moraxella bovis. (NADL) (ADP al-37)

N. The Immunizing Effect of Brucella Cell Wall (PL 480 project)

Under a PL 480 Grant, investigations on "The Immunizing Effect of Brucella Cell Wall" are in progress at the Hebrew University, Hadassah Medical School, Jerusalem, Israel. Their report shows that killed preparations derived from Br. abortus cell walls conferred immunity to mice for up to 90 days. The period following challenge during which the mice were free from infection was very short. Animals examined at 28 days or more after challenge with high doses of bacteria were found to harbor large numbers of Brucella organisms in the spleen. Neither reinfection from extraneous sites nor the multiplication of a small number of organisms in the spleen could be implicated in the phenomenon of reversion. Vaccines prepared from cell walls of Br. abortus, melitensis and suis proved more effective than intact cells. The more recent experiments with cell wall fractions indicate that further purification of the immunizing antigen is feasible.

(Israel) (AlO-ADP-6)

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FOOT-AND-MOUTH AND OTHER EXOTIC INFECTIOUS DISEASES OF CATTLE Animal Disease and Parasite Research Division, ARS

Problem. The Congress in 1948 authorized establishment of a laboratory in the United States for research on foot-and-mouth and other exotic animal diseases. The law required that the laboratory and related facilities for research and study be located on a coastal island separated from the mainland by deep, navigable waters. Plum Island was selected as the site for the laboratory on July 28, 1952. The Plum Island Animal Disease Laboratory as a U. S. Department of Agriculture venture came into existence on July 1, 1954, and since that time this laboratory has been responsible for protecting the nation's livestock industry against animal diseases of foreign origin. Foot-and-mouth disease has visited the United States on 9 occasions and each time has been eradicated. The last outbreak of foot-and-mouth disease was in 1929. Contagious bovine pleuropneumonia was eradicated in the 1880's and has not recurred since. Success in keeping these exotic animal diseases out of the United States has been due to a number of factors and a continuing vigilance by U. S. Department of Agriculture personnel.

The establishment of the Plum Island Animal Disease Laboratory and its continuing research program on exotic animal diseases has provided a laboratory in the United States where research on animal disease foreign to our soils is carried out. As new information is developed at the laboratory, it is made available to those agencies in the Department responsible for keeping out livestock animal diseases which do not occur in this country. Foot-and-mouth disease is capable of reducing our overall productivity by 25% in areas where it might become established. The disease exists in all large land areas of the world with the exception of Central and North America, Australia, and New Zealand.

Rinderpest, a disease of cattle, continues to be a serious disease problem in Africa and Asia. This disease is capable of killing 90% or more of the cattle exposed to it. Other diseases for which the laboratory is responsible include contagious bovine pleuropneumonia, Rift Valley fever, East Coast fever, and lumpy skin disease. All of these diseases continue to cause severe losses in other parts of the world. The possibilities of entry of these diseases in the United States continues, primarily because of the progressively increasing scope, speed, and extent of modern international transportation. Information developed at the Plum Island Animal Disease Laboratory is applied to the protection of the nation's livestock against foreign animal diseases.

The research continues to develop and maintain a competence for diagnosis of exotic animal diseases. Fundamental research is being conducted on biological, chemical, and physical properties of the infective agents that may be useful in prevention, control, and eradication of these diseases.

USDA AND COOPERATIVE PROGRAM

The <u>Department</u> at its Plum Island Animal Disease Laboratory has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists engaged in basic and applied research in this problem area. All of this research is conducted at the Plum Island Animal Disease Laboratory, Greenport, New York, except for supplemental field studies on foot-and-mouth disease vaccines which is conducted cooperatively in The Netherlands. The Department is also engaged in research under terms of an Interagency Agreement with the Assistance In Development Program, U. S. State Department, in Kenya, on contagious bovine pleuropneumonia.

The Federal scientific effort devoted to research in this area conducted solely at the Plum Island Animal Disease Laboratory, totals 23.5 professional man-years. This effort is divided among sub-headings as follows:

Studies on foot-and-mouth disease virus 2.0

Determine mechanism of antibody formation 1.0

Immune response of cattle to types and sub-types of foot-and-mouth disease virus 1.0

Quantity production of foot-and-mouth disease virus 2.0

Establishment and characterization of cell lines and cell strains 1.0

Mechanism of the interaction between foot-and-mouth disease virus molecules and host cells 2.0

Genetic biochemistry of foot-and-mouth disease virus 1.0

Effects of chemical and physical environment on foot-and-mouth disease virus 1.0

Bulk freeze drying of foot-and-mouth disease virus vaccine and antiserum 1.0

Identification, purification and chemical and physical characterization of foot-and-mouth disease virus and other exotic animal viruses 2.0

Immuno-chemical investigations of foot-and-mouth disease virus 1.5

Attenuation of representative types of foot-and-mouth disease virus 1.0

Survival and inactivation of foot-and-mouth disease virus in meat and meat by-products 1.0

Biological mechanism of natural resistance and susceptibility to foot-and mouth disease virus 1.0

Biological alteration of foot-and-mouth disease virus from continual residence in cell cultures 1.0

Morphological aspects of virus-cell relationships 1.0

Diagnostic and immunizing procedures for contagious bovine pleuropneumonia 3.0

Work was continued under a PL 480 grant to the Instituto Biologica, Sao Paulo, Brazil, for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus, and experimental field vaccination.

Under a PL 480 grant to the Ministry of Agriculture, Laboratories of Foot-and-Mouth Disease and Tissue Culture, Etlik, Turkey, research is under way on "Studies of Various Indigenous Types of Foot-and-Mouth Disease Virus, and the Production of a Vaccine for the Control of Foot-and-Mouth Disease in Turkey."

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Immunological Studies on Foot-and-Mouth Disease Vaccine

Chemically treated baby hamster kidney (BHK) cell culture virus used with an oil adjuvant protected steers from infection with homologous virus exposure for a six month period. The response to this inoculum in cattle, appeared to correlate the response in swine.

Foot-and-mouth disease virus degraded to small particle by acid at pH 4.8 was found to react with foot-and-mouth disease (FMD) antibody and block it to the extent it was no longer able to neutralize infectious virus. This blocking could occur with heterologous small particle (type 0) as well as the homologous type (type A). These data indicate strongly that the small particle of foot-and-mouth disease virus (FMDV) may have antigens common to all of the virus types and that specificity is associated with the intact virus particle. (ADP a8-8(R)

B. Immunological Study - Mechanism of Antibody Formation

The interaction between virus and antibody was found to be time and temperature dependent. These variables may reflect differences in neutralization values as determined by suckling mice and bovine kidney cell culture assays. Higher PD values were obtained at room temperature and at 37°C than at 0-5°C over a 6-hour period of incubation. Un-neutralized, infectious virus was detected in bovine kidney cell cultures from serum-virus mixtures that were innocuous for suckling mice. This observation may also account for the discrepancy between suckling mice and tissue culture assays.

Glycidaldehyde (GDA) was determined to possess desirable stability characteristics as compared to more popular inactivants. Little loss in virucidal potency was noted over a seven month period and after storage at ambient temperatures. A substantial amount of the virus inactivating capacity of GDA remains intact, although reduced, even after 24 hours at 37°C, while after 4 hours at 37°C, the slope of the inactivation curve was similar to that produced by freshly added GDA. A more rapid rate of inactivation by .02% GDA was noted when medium containing less GDA combining constituents was used. The overall effect of extraneous materials in the medium is to lower the concentration of GDA available for virucidal action and to decrease the rate of inactivation.

Suspensions of FMDv, A-119, propagated in baby hamster kidney cells, were treated with .02% GDA for 4 hours at 37°C. The rate of inactivation indicated a departure from first-order reaction kinetics. Two phases of inactivation were noted, but it is concluded that, with appropriate interpretation of the reaction kinetics, the inactivation does lend itself to that type from which a predictable margin of safety may be determined.

Infectious and killed virus preparations were antigenically potent and produced antibody levels capable of protecting animals against high challenge doses of infectious virus. (ADP a8-10(R)

C. Immune Response of Cattle to Types and Sub-Types of Foot-and-Mouth Disease Virus

Convalescent bovine sera have been assayed for neutralizing antibody, using suckling mice and tissue culture as the assay system. Values for levels of neutralizing antibody have been consistently higher when assayed in mice than when assayed in tissue culture. Differences in the rate of inactivation of virus by sera taken at different times following infection, appears to be attributable to the type of antibody present in the serum.

Knowledge of the protection conferred by foot-and-mouth disease vaccines and understanding of available methods for evaluating the degree and duration of such immunity are essential for planning of field vaccination programs. Information can only be obtained by observations and study involving large numbers of cattle over long periods of time. An ADP representative has been conducting investigations in cooperation with Dutch Ministry of Agriculture scientists in The Netherlands where annual vaccination of cattle against types 0, A, and C foot-and-mouth disease is practiced. Cattle for serological and infectivity studies are available from selected Dutch dairy herds, from certain groups of animals destined for slaughter at the Amsterdam abattoir and animals used for vaccine potency tests at the Central Veterinary Institute.

Serum antibody titers against the three types of virus persisted over a four-year period in most of the 360 cattle which had been vaccinated two or more times at annual intervals. Antibody levels remained relatively

constant from 12 or 18 months following vaccination through a four-year period. Average antibody levels against type C virus were considerably lower than against types O and A.

In general, a good correlation was observed between serum antibody level and resistance to infection. In studies using type C virus, animals challenged two weeks following vaccination were more resistant than those with comparable titers exposed 9 to 48 months post-vaccination. (ADP a8-11(R)

D. Quantity Production of Foot-and-Mouth Disease Virus

Studies were continued to develop basic information on virus-cell interrelationships applicable to better methods for detection, assay, and production of virus in cells grown on glass and in suspension.

Strains of all 7 types of foot-and-mouth disease virus (FMDV) were characterized according to frequency distribution of plaque diameters. There was a significant relationship between plaque size in primary cultures of bovine kidney cells and infectivity for cattle by intramuscular (i.m.) inoculation. Low dosage levels of large plaque strains were superior to small plaque strains for infecting cattle by the i.m. route. No relationship existed between plaque size and infectivity when the same strains were inoculated into mice, guinea pigs, cattle, or cell cultures. An inverse relationship existed between number of plaques formed by FMDV in primary bovine kidney cell cultures, and the concentration of bovine, young calf, or agamma newborn calf serum was used in the growth medium. Fewer plaques were formed when change medium contained bovine serum.

There was no evidence of reduced susceptibility of primary cultures of bovine kidney cells to infection with FMDV when serum containing antibodies against FMDV was used in the growth medium, providing the cell cultures were washed at time of medium change and the change medium was free of serum. Failure to wash the cultures resulted in reduction of number of plaques in cultures inoculated with virus homologous to the serum.

(ADP-a8-12(R)

E. Establishment and Characterization of Cell Lines and Cell Strains

Plaque formation by different strains of FMDV in a line of baby hamster kidney cells is being studied.

Studies on susceptibility of a line of pig kidney cells to infection with various strains of FMDV have been initiated. (ADP a8-14(R)

F. Mechanism of the Interaction between Foot-and-Mouth Disease Virus Molecules and Host Cells

Chemically-defined medium for baby hamster kidney cells (BHK). were made on the development of a protein-free, chemically-characterized medium for the growth of baby hamster kidney culture cells on glass for use in the study of the biosynthesis of FMDV. In high multiplicity infection experiments in BHK cells of 5-hour duration, no definite medium was as satisfactory as the original growth medium containing serum and lactalbumin hydrolyzate. TB-H, glu, fortified with 0.03% glutamine, allowed the production of 0.5 log units less virus infectivity than complete growth medium, and was the best defined medium to be developed thus far. In media studied, the virus produced within 5 hours was still largely intra-cellular. Glutamine was not replaceable by lactalbumin hydrolyzate, nor by substances known to be produced from it in vivo, i.e., glutamic acid, proline, aspartic acid, asparagine, and the RNA bases uracil, cytosine, adenine, and guanidine. Glutamine stimulated virus production independently of the action of glucose, each chemical yielding about 1.2 log units less virus than complete growth medium. Glutamine, unlike glucose, stimulated oxygen uptake in uninfected cells by an average of 23%. In infected cells, which showed decrease in oxygen uptake, glutamine was able to enhance respiration. apparent maintenance of cellular viability by glutamine may help explain its role in increasing viral production.

Studies were continued on the effect of chemical agents on FMDV production in cell cultures. Amantadine was tested against FMDV in baby hamster and calf kidney cell cultures. Concentrations of 25 and 250 µg/ml of agent in growth medium containing lactalbumin hydrolyzate and serum were ineffective in reducing virus yields in experiments at a high multiplicity of infection during 5 hours, and in low multiplicity experiments during 18 hours. Amantadine was ineffective in the absence of lactalbumin hydrolyzate and serum in the growth medium in high multiplicity of infection experiments, but did inhibit virus production when infection was at a low multiplicity. This inhibition could be attributed to loosening of cells from the glass surface by the toxicity of the chemical. (ADP a8-17(R)

G. Studies on Genetic Biochemistry of Foot-and-Mouth Disease Virus

Control of DNA function in cells infected with FMDV. Infection of animal cells with viruses has been shown to shut off normal cellular transcription. It was decided to examine whether this regulation could occur by a histone pathway. The histones of baby hamster kidney cell cultures were examined before and after infection with FMDV, A-119, for changes in heterogeneity, methylation, acetylation and amino acid incorporation. It was found by electrophoretic analysis that infection by FMDV rapidly increased the heterogeneity of cells with arginine rich histones. This suggested an increased histone control mechanism in infected cells. Similar changes were observed by extensive passage of noninfected cells. In support of a histone pathway for decreasing DNA transcription in infected cells, it was

observed that ¹⁴C lysine and ¹⁴C arginine were incorporated at a 50% greater rate into both lysine and arginine rich histones immediately after infection. Likewise, increased regulation by histone after infection was revealed by 3.5-fold decreases in the rates of acetylation (by ¹C sodium acetate)₁Qf the N-terminal group of the arginine rich histone and methylation (by ¹C methyl methionine) of the e-amino group of the lysine-rich histone.

Reaction of FMDV with Cations and Formaldehyde. Structural changes during the reaction of pure FMDV with cations and formaldehyde were deduced from ultraviolet absorbance measurements. Absorbance-time and absorbance-temperature profiles of FMDV were determined sequentially in the presence of sodium and magnesium ions and in CH₂O. It could be inferred from the profiles that FMDV broke down spontaneously at 10°C in O.001 M Na⁺ to protein and hypochromic ribonucleic acid. The latter then denatured reversibly when heated. Mg⁺⁺ at 10⁻³ markedly suppressed the spontaneous degradation of FMDV; lower concentrations of Mg⁺⁺ were progressively less effective. CH₂O at 0.25% appeared to cause the degradation of FMDV at 10°C at a sodium ion concentration, O.02 M, where the virus was otherwise stable up to 53°C. CH₂O did not lower the degradation temperature as much at higher concentrations of sodium ions where the virus was known to be more stable. Plausible mechanisms were suggested for the action of heat, cations and CH₂O on FMDV. (ADP a8-18(R)

H. Effects of Certain Chemical and Physical Environments on Foot-and-Mouth Disease Virus

A 1 M concentration of hydroxylamine reduced the titer of FMDV 0-Mll 6-7 logs in 15-30 minutes at temperatures of 37, 23, and 4°C. The titer was reduced to the same extent by a 0.1 M concentration of the chemical at 4°C, but 28 hours incubation was required. Better immunological preparations, as tested in adult chickens and mice, were obtained when 0.25 M concentrations were used at 23 or 4°C for 6 to 18 hours incubation.

Argentine strain A-l FMDV was inactivated with 15 minutes by 6 μ g./ml. concentrations of methylene blue, neutral red and toluidine blue followed by exposure to light rays of incandescent bulbs at a temperature of 15°C. With crystal violet used similarly, there was only a loss of about 2 logs of virus titer. The methylene blue treated virus was more immunogenic than the others.

A comparison was made between cattle strains of FMDV and their counterparts after adaptation to cell cultures regarding the stabilizing action of divalent cation, Mg++, at 2 M concentration and temperature of 50°C. The laboratory cattle strain, C-149, was very stable under this treatment and Argentine field strains of types A, O, and C were not as well stabilized. The cell culture adapted viruses from these four strains were entirely unstable with this treatment. This technique may serve as a means of inactivation of cell culture adapted viruses and a means for differentiating such viruses from field or laboratory strains.

At a concentration of 0.05%, acetylethyleneimine (AEI) inactivated FMDV and allowed retention of immunogenic properties. Similar treatment with beta propiolactone left a residuum of active virus. However, when the latter treatment was preceded by ultraviolet irradiation, inactivation of virus resulted and immunogenic properties were retained comparable to AEI treatment.

Foot-and-mouth disease virus, placed in an antiserum of another type as a simulated contaminant, was inactivated by the addition of 0.3% beta propiolactone. After this treatment, the antiserum retained its neutralizing antibody activity, but there was a three tube loss in complement fixing antibody activity.

The effect of 50, 5 and 0.5 µg. of glutaraldehyde on bovine kidney cell cultures was determined. Some toxic effects, as indicated by rounding of cells, were noted due to 50 µg. of the compound but no toxic effects were produced by 5 or 0.5 µg. of glutaraldehyde. No adverse effects were noted on virus multiplication at these three levels. Glutaraldehyde inactivated approximately 3 log units of FMDV at .05% but only 1 to 0.5 logs of virus at .005% and .0005% after 4 hours at 37°C, respectively. Further studies are necessary to fully evaluate this compound. (ADP a8-19(R)

I. Bulk Freeze-Drying of Foot-and-Mouth Disease Virus Vaccines and Antiserums

A study was made of freeze-drying conditions, using cell culture adapted strain A-119 FMDV with various supporting additives as the test agent. The drying conditions that gave the better results were: A residual air pressure of <100 microns, temperature of product at 22 C and condenser at -50 C, a drying time of 36 hours and a product volume of 4.0 ml. Under these conditions, dried virus stored at 4 C in flame-sealed ampules retained full infectivity for one year. The cell culture maintenance fluid was as effective in preserving virus as the additives: skim milk, sucrose, sodium glutamate or normal cattle serum. (ADP a8-20(R)

J. Identification, Purification and Chemical and Physical Characterization of Foot-and-Mouth Disease Virus and Other Exotic Animal Viruses

A. Electron Microscopy. African swine fever virus (ASFV) was grown in a stable swine-kidney cell line, and electron micrographs of thin sections of infected cells were made during various stages of viral development. Two hours after infection virus was seen within the cytoplasma. These particles appeared to be those which had been taken up from the inoculum, since no evidence was seen of virus reproduction. In subsequent thin sections taken 24, 48, 72, and 96 hours after inoculation, areas of virus formation appeared and increased in size until the cytoplasm disrupted. A few virus particles were seen in the intercellular spaces after 24 hours, while later micrographs showed many particles budding out through the cell wall. By 96 hours, a large portion of the cytoplasm had been converted to

virus, and the cell disintegrated. The process of virus release was a continuous rather than burst process. During release the particle acquired an outer membrane of cellular material. The structure of fully developed ASFV was unique in processing a very osmophilic hexagonal wall which surrounded an electron lucent region and a central nucleoid. Measurements across the virus and nucleoid ranged from 175-215 mu and 72-89 mu, respectively. The period during which ASFV particles were forming in the cell was consistent with the rise of infectivity and hemadsorptinin in cell culture fluids.

FMDV structure. The rotational technique of Markham et al was used to compare electron micrographs of highly purified FMDV with rotational photographs of two models most likely to represent the structure of FMDV. The purpose was to determine more accurately the capsomeric structure of FMDV. One model was a 32 subunit rhombic tricontahedron, while the other was a 42 subunit icosahedron. A comparison of the rotational images showed that the 32 and 42 structural unit models were not readily distinguished in most instances. What might be called secondary effects of the reinforcements did indicate, however, that FMDV images were more consistent with the 32-unit structure than they were with the 42-unit structure.

Gamma Irradiation of FMDV. Work has been completed on the gamma irradiation of foot-and-mouth disease virus, type All9, from baby hamster and calf kidney tissue cultures. Comparisons were made on the effect of the cobalt-60 exposure on both crude and pure virus. Virus was maintained at 0°C except during exposure in the 60 Co source where the temperature remained cold to the touch even after a 60 minute exposure. The change in the infectivity of both preparations of virus was monitored as well as the physical state of the pure virus as determined by ultraviolet absorbancetemperature profiles. The approximate intensity of the 60co source was 3400 rads/min. at the sample location. Virus infectivity showed appreciable resistance to gamma rays only when protective substances were present. Inactivation rates of crude and pure viruses were approximately 0.4 and 7.0 log units/hr., respectively. Addition of gelatin (0.1%) or cysteine (0.1%) did not stabilize crude virus any further, but caused pure virus to be nearly as stable to gamma rays as crude virus. From knowledge about ionizing effects of gamma rays in aqueous systems, it appears that the additives functioned by neutralizing the newly-formed free radicals and peroxides.

Hydroxymercuribenzoate (HMB) had no effect on either crude or pure FMDV. In contrast to the apparent enhancing effect of cysteine on the infectivity of non-irradiated pure virus, HMB appeared to cause some inactivation, indicating that sulfhydryl groups in the virus may be important to infectivity. From absorbance-temperature profiles on irradiated pure virus, it could be inferred that considerable degradation of virus occurred within 1 minute of exposure. At 10 minutes, it could be deduced that scission of the sugar-phosphate backbone structure of viral RNA had commenced. At 30

minutes, backbone scission was nearly complete, and destruction of purine and pyrimidine rings had commenced. At 60 minutes, both kinds of breakage appeared to be complete.

Amino Acid Composition of FMDV. Analysis has been made of the amino acid composition of FMDV, types A_{119} , 0_9 , and C_3 produced in baby hamster kidney cultures and purified by procedures developed previously. Analysis was also made of type A_{119} virus from cattle which had been passaged only a few times in calf kidney tissue cultures. Statistical comparisons revealed no differences at the 0.05 level of significance in the amino acid content of type A_{119} virus whether grown in baby hamster or calf cells. However, type 09 virus differed from type A_{119} in its content of alanine, leucine, tyrosine and possibly histidine. Type C_3 differed in its content of threonine, serine, alanine, valine, isoleucine, tyrosine, phenylalanine, lysine and possibly tryptophan. Type 0_9 and 0_7 differed from one another in their content of all the amino acids listed previously, as well as in glycine and arginine and possibly 1/2 cystine.

Application of Digital Computers to Ultracentrifugation An investigation of the applicability of digital computers to analytical ultracentrifugation has been completed. Since Plum Island does not possess a computer, the facilities of other institutes both in the United States and England, were employed. Three major problems were examined which apply to virus research: a) determination of sedimentation coefficients from data of radial position of the solute as a function of time; b) determination of the molecular weight from solute redistribution data, and c) determination of interaction constants from summary data of sedimentation coefficients as a function of concentration. It was concluded that a) ultracentrifuge calculations which are too tedious to compute manually can be computed automatically; b) digital computers permit assessment of internal errors and the building in of safeguards; c) both linear and non-linear least squares statistics can be used, and d) certain specified criteria should be applied when using (ADP a8-25) computer programs developed by others.

K. Immuno-Chemical Investigations of Foot-and-Mouth Disease

Cattle infected with foot-and-mouth disease virus produce four, and possibly five different molecular species of antibodies. The antibody detected first is a 19S y_1 -globulin that reaches a high level by the 7th day but is not readily detectable after about 30 days. By the 14th day, antibodies are present that have lower sedimentation rates. These have been fractionated into three or four different electrophoretic classes of antibodies. One of these antibodies is the 7S y_2 type and it did not fix complement as well as the faster migrating antibodies. The persistence of the different antibody types is apparently dependent upon the method of exposure to virus antigen.

Conditions for testing bovine and swine serum by the complement-fixation technique were established. (ADP a8-26)

L. Survival and Inactivation of Foot-and-Mouth Disease Virus in Meat and Meat By-Products

A study of the survival of FMDV in cattle hides was prompted by the great numbers of hides that are annually imported from FMD countries. Experimentally infected cattle were slaughtered at various times after inoculation and hide samples were taken from shoulder, lumbar, inner thigh and perineal regions. The samples were shaved and aseptic precautions were taken. With all seven types of FMD, virus was regularly detected in fresh hide samples taken from 32 cattle during the period of viremia. From 6 to 28 days after inoculation, virus was found in hides of 14 of 22 cattle, with the longest persistence of 18 days. The highest virus titer found in hides was 10⁵ · 5 cell culture plaque-forming units per gram (PFU). There was no significant difference in titers of virus from the various skin areas. A study of FMDV survival in dried, salted and chemically treated hides is in progress.

The importation of various glands and tissues from FMD countries for production of biological products prompted a study of the survival of virus in the central nervous system structures. Foot-and-mouth disease virus was detected in high titers in the pituitary gland of experimentally infected cattle from the early clinical to the early convalescent stages of the disease. Virus persisted for as long as two days after viremia ended. The highest titer obtained was $10^{6.8}$ cell culture PFU/gm. and the virus titers in the pituitary were equal to or higher than those found in the blood.

Virus was also isolated from the spinal cord, pineal body, cerebrum and cerebrospinal fluid, but less frequently and, with lower titers than from the pituitary. Virus was not isolated from cerebellum, medulla or hippocampus. Additional studies are in progress on survival of virus in other endocrine glands.

(ADP a8-28)

M. Studies on the Biological Mechanisms of Natural Resistance and Susceptibility of Foot-and-Mouth Disease Virus

Mice from litters consisting of 5 and 10 animals and therefore of different weights although of the same age, were equally susceptible to FMDV at 7, 14, and 21 days of age. Their response at 21 days of age indicated that some litters of mice were more susceptible than others. Extension of this work to 28- and 35-day-old mice revealed that the mice of certain litters were still susceptible while those from other litters were resistant to the virus inoculum used.

Efforts were made to learn if a relation existed between in vitro virus production by mouse kidney cells and the in vivo response of the cell donor. Single kidneys were surgically removed from adult mice, and virus production was determined in suspensions prepared from the kidneys. The recovered mice were inoculated with virus and their response was observed. A meaningful correlation between the amount of virus produced in vitro and the animal's response was not obtained, due at least in part to the fact that

the mice were more susceptible following the operation. Similarly, comparison of <u>in vitro</u> virus production with the response of litter mates of the cell donors also failed to show a consistent pattern perhaps due to the variation in response of 35-day-old mice.

Inoculation of suspensions of minced kidneys from 7- and 35-day-old mice resulted in adsorption of 80-90% of the virus over a 3 to 4 hour period regardless of the virus concentration at the start. To date, the experiments indicate that 10-20% of this virus population is a variant which is not adsorbed by these cells under the experimental conditions.

In the performance of the plaque assay, incubation of cultures with confluent cell sheets at 37, 30, or 24°C before inoculation is satisfactory if a 37°C temperature is provided during the adsorption and subsequent incubation period. Rotation after 30 minutes of adsorption is unnecessary. A 2% serum concentration in the overlay medium is as satisfactory as 10% for plaque formation. (ADP a8-29)

N. Biological Alterations of Foot-and-Mouth Disease Virus from Continual Residence in Cell Cultures

Foot-and-mouth disease virus type A, strain 119 that had undergone primary modification by chronic residence in primary calf kidney cell cultures had no practical value as an immunizing agent. Subclinical infection required to produce immunity in cattle occurred regularly only when small carefully regulated doses were given. Larger doses sometimes failed to produce subclinical infection on account of an interference factor in the virus, or produced mild clinical signs of the disease.

Significant improvement was obtained by secondary modification of the virus effected by a single passage in cattle. The secondary modified viruses were obtained from the blood of cattle with subclinical infection from the primary modified virus. One secondary modified virus consistently produced subclinical infection (viremia without clinical signs of the disease) in a wide range of dosage. Cattle inoculated with this virus resisted challenge with virulent foot-and-mouth disease virus 22 days later.

Lyophilization damaged the secondary modified virus to the extent that some inoculated animals developed signs of the disease and some failed to become immune.

Doses of these modified viruses that produced subclinical infection in cattle, produced clinical infection in swine, although the disease was mild as compared with natural infection.

(ADP a8-30)

O. Morphologic Aspects of Virus-Cell Relationships

Growth rates of cell "lines" developed from cell cultures surviving infection with FMDV were apparently less than those of conventional type cell lines. One such "line" was obtained from primary swine kidney cultures and one from primary culture of a canine seminoma. Their status (primary or permanent) remains to be determined.

Three types of unmodified FMDV (A-119, C3 CANEFA and O-M11) were propagated in primary canine kidney cell cultures. Low titer virus yields indicated intermediate degree of cell susceptibility to the viruses. Higher titers of canine cell passaged viruses were obtained by subsequent passage in primary lamb testis cell cultures. (ADP a8-31)

P. Diagnostic and Immunizing Procedures for Contagious Bovine Pleuro-pneumonia

The value of mice and cell cultures for propagation of the etiologic agent has been investigated. In both systems, Mycoplasma mycoides will reproduce and persist but neutralization of the activity has not been successful. M. mycoides appears to be extremely sensitive to ethylene oxide gas as the growth of cultures were inhibited in media previously exposed to the gas.

A variety of diagnostic and reference materials have been produced for assistance in diagnosis of contagious bovine pleuropneumonia. These materials consist of - rabbit-immune serum, diagnostic antigens, and experimental serums produced in guinea pigs.

Work has also been conducted to develop a fraction of the organism which, when inoculated into cattle immune to contagious bovine pleuropneumonia, will cause a delayed skin reaction. The development of such a method would assist, immeasurably, in arriving at a diagnosis. (ADP a8-32)

Q. Studies on Foot-and-Mouth Disease Virus (PL 480 project)

Under a PL 480 Grant, research is being conducted on foot-and-mouth disease virus (FMDV) at the Instituto Biologica, Sao Paulo, Brazil. During a 6-months period 27 samples were collected from spontaneous cases of foot-and-mouth disease. Thirteen were positive in direct complement fixation tests. Types isolated were A, O, and C. Investigations on comparative observation on bovine and swine kidney cell cultures revealed that swine primary cultures are always highly susceptible, and the bovine cultures usually presented variable response to the virus infection. Doses which induced an extensive cytopathic effect in "normal" primary bovine cultures, produced only focci of dead cells in the resistant ones. Studies on 5 long-term bovine cultures showed that they had lost most of their primitive susceptibility to FMDV, and they had a diploid number of chromosomes and a low growth rate. Long term observations on 3 swine cell lines showed they had maintained their susceptibility to the same virus. (E3-ADP-2)

R. Studies on Various Indigenous Types of Foot-and-Mouth Disease Virus, and the Production of a Vaccine for the Control of FMD in Turkey

(PL 480 project)

Under a PL 480 Grant to the Ministry of Agriculture, Laboratories of Footand-Mouth Disease and Tissue Culture, Etlik, Turkey, research was conducted on various types of foot-and-mouth disease virus indigenous to Turkey, and on the production of a vaccine for the control of the disease in Turkey.

SAT 1 Ova. F4-Kn9 strain virus was obtained from sheep. This SAT 1 type of foot-and-mouth disease infection was widespread in Turkey. It was easy to adapt to sheep. Work on other strains of the virus was stopped for the present. Ubetinis' method was found to be satisfactory for trypsinization of kidney tissue cells. Serums from horses, healthy unvaccinated cattle and recovered cattle were found to be satisfactory for use in calf and lamb kidney cell cultures. The entry of Type A FMD virus from Iran resulted in a delay of the work on SAT 1 until new facilities could be established. The cell culturing studies are progressing satisfactorily. (A22-ADP-8)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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PARASITES AND PARASITIC DISEASES OF CATTLE Animal Disease and Parasite Research Division, ARS

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of \$400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 19.5 professional man-years. This effort is divided among subheadings as follows:

Ecological Factors Influencing Gastro-Intestinal Nematodes of Cattle 1.0 at the Animal Disease and Parasite Research Division, Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effect of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Disease Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Acquisition and Effects of Roundworm Parasites of Cattle as Influenced by Diet 1.0 at the Animal Disease and Parasite Research Division, Beltsville Parasitological Laboratory, Beltsville, Maryland.

Host-Parasite Relationship of Coccidial Parasites of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Ecology and Immunology of the Cattle Lungworm, Dictyocaulus viviparus 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, Including Anthelmintic Treatment 1.5 at the University of California, Davis, under a cooperative agreement with the ARS-USDA.

<u>Investigations of Trichomonad Parasites</u> 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan, Utah.

Host-Parasite Relationship of Intestinal Worms, Cooperia spp. in Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Etiology and Immune Response of Cattle to Winter Coccidiosis 1.0 at the Regional Animal Disease Laboratory, Logan, Utah, and under a cooperative agreement with the Montana Agricultural Experiment Station, Bozeman.

Anaplasmosis of Cattle 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and through a Memorandum of Understanding and other agreements in cooperation with the State Experiment Stations in California, Illinois, Louisiana, Nevada, and State Veterinarian of Tennessee, the USDA Entomology Research Station, Kerrville, Texas, and the Delta Branch Experiment Station, Stoneville, Mississippi.

Interrelationships of Diet and Parasitic Infection in the Production of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Histochemistry of Gastro-Intestinal Nematodes of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Parasites of Cattle with emphasis on Stephanofilarial Species 1.0 at the Animal Disease and Parasite Research Division Regional Animal Disease Laboratory, University Park, New Mexico.

Effect of Stocking Rate and Rotational Grazing on Internal Parasitism of Cattle 1.0 at the Regional Animal Disease Laboratory, Auburn, Alabama.

Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (Cattle, Sheep, and Alpacas) (PL-480 Peru)

Investigations on Anaplasmosis, Piroplasmosis and Babesiallosis of Cattle are under way through a PL 480 Grant at the School of Veterinary, Montevideo, Uruguay (PL 480 Uruguay)

PROGRAM OF STATE EXPERIMENT STATIONS

The State Stations have a long term program covering basic and applied aspects of the major internal parasite problems of cattle. Twelve states in the Western Region and the Department are cooperating in regional research on cattle nematode problems (W-35). Informal coordination is maintained with States in the Southern Region also working in this subject matter area.

Basic research is in progress to establish how nematodes damage the host animal, interfere with nutrition and result in disease. Research on the biochemical systems concerned with parasite metabolism and the effect of anthelmintics on these systems is providing basic information necessary in developing improved therapeutic controls. Other studies are centered on means for reducing the exposure of cattle to infective stages of parasites. Systems of grazing management and feeding procedures are being evaluated and factors which favor over-wintering survival of parasite larvae are being determined. The relationship between types of pasture forage and the degree of parasitism are being determined and the micro-climatic conditions conducive to parasite larval infectivity are being established. Immune mechanisms involved in resistance to parasites are being determined.

A number of states are engaged in studies on the nature of Anaplasma, the life cycle of this parasite, methods for immunization against anaplasmosis and procedures for eradicating the disease. Other work is in progress on the life cycle and means of controlling coccidiosis of cattle.

The total State scientific effort devoted to this research is 29.1 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Etiological Factors Influencing Gastro-Intestinal Nematodes of Cattle

The report on research conducted at Experiment, Georgia, under the auspices of the Regional Animal Disease Laboratory at Auburn, Alabama, shows the use of vermiculite as a culture medium for larvae of nematodes of ruminants was investigated. Feces containing eggs of Oesophagostomum radiatum, Cooperia pectinata, C. oncophora, Trichostrongylus colubriformis or T. axei was collected from monospecifically infected calves and cultured in either the conventional sphagnum moss or vermiculite (Terra-Lite Brand, Zonolite Company). Average larval recovery was higher from cultures made with vermiculite than those made with moss, although the differences were not significant. Larvae in sheep pellets were also cultured successfully with vermiculite. In general, vermiculite was more advantageous for routine culturing, being cleaner, more hydrophilic, more economical, and conveniently packaged. Furthermore, the granular nature of vermiculite makes it easier to mix and spread out on the water surface of a Baermann funnel. (ADP bl-6(R)(Alabama and Georgia)

B. Host-Parasite Relationship of Coccidial Parasites of Cattle.

Scientists at the Animal Disease and Parasite Research Division's Regional Animal Disease Laboratory at Auburn, Alabama, reported the following: When 20% antiformin and 1.0 M sulfuric acid were used consecutively or separately there was very little reduction in sporulation or destruction of oocysts of Eimeria ellipsoidalis, E. auburnensis, and E. Bovis, but when the two solutions were combined, the mixture nearly completely prevented sporulation and destroyed almost all of the oocysts and contents. Half-strength solutions were not as effective, as there was no evidence of destruction and all oocysts were not prevented from sporulating. Half-and quarter-strength solutions were more effective in reduction of sporulation of oocysts of E. auburnensis than oocysts of E. bovis.

Additional data were obtained on the life cycle of Eimeria cylindrica in calves. Six young calves were inoculated with 50,000 to 240,000 occysts of E. cylindrica to determine the prepatent period. Five of the six became infected and had occyst outputs ranging from light to heavy. The prepatent period was 9 days postinoculation (PI) in one, and 10 days in the other four. Peak days of occyst discharge were 12, 12, 19, 13, and 10 days PI. Patent periods lasted 8, 5, 21, 13, and 12 days. Data were obtained on the endogenous stages of the parasite in three other calves, two killed at 8 days PI and one killed at 11 days. In the first calf killed at 8 days, macrogametocytes were found in the crypts of Leiberkuhn in the lower small intestine. In the other calf killed at 8 days PI, immature macrogametocytes measuring up to 19.5 µ were found in the last portion of the small intestine in the glandular crypts.

Two calves inoculated with oocysts of <u>Eimeria canadensis</u> were killed at 9 and 16 days PI to obtain information on the endogenous stages of the life cycle. At 9 days PI, immature schizonts were found in the small intestine in the apices of villi one foot below the stomach and at 24 ft. above the ileocecal valve. At 16 days PI, merozoites ranging up to 18.2 µ were found in great numbers from C plus 42 ft. to C plus 6 feet. Schizonts were most numerous at C plus 12 to C plus 24. They were macroscopic and were located in the lacteals of the villi, resembling those of <u>E. bovis</u> and <u>E. auburnensis</u>. Microgametocytes resembled those of <u>E. auburnensis</u>; the microgametes were arranged peripherally and invaginated at places.

(Auburn, Alabama) (ADP bl-23(Rev.)

C. Clinical and Physiological Aspects of Roundworm Parasitism in Cattle including Anthelmintic Treatment.

The School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, reports on their investigations as follows:

Anthelmintic Studies (a) Disophenol (DNP), an excellent anthelmintic against a narrow spectrum of nematodes, was found to have significant action against Fasciola hepatica, the common liver fluke of sheep and cattle. (b) Haloxon at 50 mg/kg in both sheep and cattle was found to remove greater than 90% of the more important species of gastrointestinal parasites in California. (c) Field trials with Thibenzole indicated that a dosage of 3 grams/100 lbs. was equal to that of 5 grams/100 lbs. in cattle as a prophylactic treatment.

Physiological Studies (a) Water balance studies indicated a marked (50%) reduction in water intake and output of cattle suffering from gastro-intestinal parasitism. It was further noted that a much greater reduction (dehydration) of the extracellular water compartment occurs than is indicated by the plasma volume.

Development of an experimental model for laboratory study of physiological alterations. Data so far collected indicate that Obellscoides cuniculi infection in the rabbit may be valuable as a model for studying many of the alterations which occur in cattle as a result of gastrointestinal parasitism. (California) (ADP b1-25)

D. Investigations on Trichomonad Parasites.

Studies conducted at the Division's Regional Animal Disease Laboratory at Logan, Utah, included the production of antisera in rabbits to six strains of Trichomonas foetus. Antisera was produced in fiscal year 1964 to two strains of T. foetus, and in 1965 to four additional strains which were acquired from widely divergent sources in this country and in Europe. The antisera were produced by two graded series of five intravenous inoculations of live, washed organisms given twice weekly with a three-week interval between the two series.

Quantity of agglutinating antibody was determined by exposing live, washed trichomonads to various dilutions of the antiserum in Ringers solution. Each trichomonad was tested against its homologous antiserum and against each of the other five antisera, and each antisera was tested against its homologous trichomonad and each of the other trichomonads. Homologous titers were 20480, 20480, 5120, 5120, 2560, and 1280 which, with the exception of the 1280 titer, indicates good strong reactions. Heterologous titers ranged from 160 to 20480.

Preliminary analysis of the agglutination results indicates there are antigenic differences in the various strains of T. foetus.

Gel diffusion studies were made comparing the six strains by the Ouchterlony gel diffusion technique. The suspended particulate material in the antigen varied in size, and some of the crude preparations plugged the gel preventing migration of antigens and subsequent formation of precipitin lines.

After centrifugation of the antigen at 37,000 gravities, an antigen of considerably better quality was produced. With the improved crude antigen, precipitin lines formed indicating the presence of four to seven antigenantibody systems. Use of the improved antigen has shown a varying number of precipitin lines form by reaction of the medium ingredients and bovine serum which is in the medium with the antiserum.

Tests made on bulls from a locality in Utah which has been troubled by trichomoniasis for the past seven years and with which we have worked closely, revealed no trichomoniasis at the present time. (Logan, Utah) (ADP bl-26)

E. Host-Parasite Relationship of Intestinal Worms, Cooperia species, in Cattle.

Reported research from the Division's Regional Animal Disease Laboratory, Auburn, Alabama, showed that cattle may be immunized against pathogenic worm parasites by controlled inoculations with closely related species. Three species of Cooperia--C. punctata, C. pectinata, and C. oncophora-are common parasites of cattle. The latter species is relatively non-pathogenic to calves, whereas the other two species are equally harmful. Calves inoculated with C. oncophora have become almost completely immune to challenge inoculation with C. pectinata and partially immune to C. punctata. Individual animals, however, failed to become immune to subsequent challenge with the original species and these calves were also unable to resist challenge with the related species.

(Auburn, Alabama) (ADP b1-27)

F. Epizootiological and Ecological Investigations of the Internal Parasites of Grazing Cattle.

Scientists at the Beltsville Parasitological Laboratory reported that malnutrition, experimentally produced in calves from 12 to 17 weeks of age, prior to infection with gastrointestinal nematodes and resultant stunting, influenced the level of parasitism acquired by them while grazing pastures, as indicated by higher worm-egg counts than those of similarly exposed full-fed calves. The driest summer on record complicated the interpretation of subsequent postmortem data insofar as determining the effects of malnutrition on the development of nematode parasitism. However, it presented an unusual opportunity to study the epidemiology of the parasitism in question under drought conditions.

Heavy contamination of the pastures with manure containing large numbers of nematode eggs during the drought led to the development of very heavy concentrations of infective larvae on the forage during the cooler weather of early fall which followed a short period of normal rainfall. The continued grazing of these heavily contaminated areas during the mild but moderately dry conditions of late fall led to extremely heavy infection of the cattle with an average of 249,000 worms per animal. The predominant

species was <u>Ostertagia</u> <u>ostertagi</u>, the medium stomach worm. This finding indicated that its free-living stages were able to survive conditions that were lethal to those of other species.

The ability of eggs of the beef tapeworm (<u>Taenia saginata</u> of man) to cause formation of cysts (<u>Cysticercus bovis</u>) in the muscles of cattle apparently was reduced rather markedly by exposure to 50,000 r, 100,000 r and 200,000 r of x-rays and in proportion to the amount of exposure. An animal that had been vaccinated with eggs exposed to 200,000 r, and was given a large dose of normal eggs subsequently, was found to be free of cysts. An average of about 3,300 cysts developed in two unvaccinated controls that received an identical dose of normal eggs. (Beltsville, Maryland) (ADP b1-28)

G. Etiology and Immune Response of Cattle to Winter Coccidiosis.

Reports on research conducted at the Division's Regional Laboratory at Logan, Utah, show that six experiments were conducted which involved coccidial infections with <u>Eimeria bovis</u> or <u>Eimeria zurnii</u> in Holstein-Friesian calves. One experiment dealt with the effect of prolonged low-level inoculations with sporulated oocysts of <u>Eimeria bovis</u> on the development of immunity in calves. Ten, 100, or 15,000 oocysts/day were given calves for 60 days. Clinical signs were exhibited only in calves receiving 15,000/day. The highest oocyst discharge also occurred in this group, but the number of days oocysts were discharged was about the same in all groups. All groups of calves exhibited resistance to reinfection when challenged with 500,000 oocysts at the end of 60 days inoculation.

Three experiments involving calves inoculated with <u>Eimeria zurnii</u> were completed. All were designed to gain information on methods of producing reliable experimental infections. Cortisone injections were used unsuccessfully in an attempt to suppress the immunogenic mechanism in the calf and allow the coccidiato invade the host. The sporulated oocysts were treated in such a way that the exterior wall would be dissolved, thus making the oocyst more susceptible to digestive fluids, including enzymes. This technique was also unsuccessful.

In the second experiment both sporulated and unsporulated oocysts were irradiated at 10,000 r, 50,000 r, 75,000 r, or 100,000 r in a cobalt-60 source. In the first part of this experiment inoculation of calves with sporulated oocysts produced results similar to those reported last year and to those reported above. However, the results were more conclusive. A challenge inoculation after recovery from the first inoculation indicated that immunity against reinfection was present in the calves which had previously exhibited clinical signs and discharged oocysts, i.e., calves receiving oocysts irradiated at 10,000 r. Little or no resistance was observed in the other 3 groups of calves. These results indicate that irradiated oocysts have no special value as immunological agents against coccidiosis caused by <u>Fimeria</u> bovis. (Logan, Utah)

Studies in cooperation with the Utah State University at Logan are reported as follows:

Description of the sporulated oocysts and sporozoites of four species of bovine coccidia. The sporulated oocyst is the stage of the coccidial life cycle most favorable for identification as to species. The bovine coccidia have been described only incompletely with respect to this stage. The description, including drawings, have been completed of the sporulated oocysts and free sporozoites of the four species most common in the Logan area, namely, E. bovis, E. zurnii, E. ellipsoidalis, and E. auburnensis. The free sporozoites were obtained for observation by causing the oocysts to excyst in vitro. The sporozoites of E. zurnii were found to have only one relatively small refractile granule in each sporozoite instead of two relatively large refractile granules as in each of the other three species. This morphological difference may be associated with the peculiar epidemiological pattern exhibited by E. zurnii, for example, its association with "winter coccidiosis" and the difficulty of inducing experimental infections with this species.

Cytological study of coccidia. A cytological study of the stages of Eimeria bovis and other bovine species of coccidia was undertaken to obtain fundamental information useful in developing methods of prevention and control of coccidia. It was necessary to section oocysts because of the impermeability of their wall. Work has demonstrated its feasibility.

First-generation merozoites of Eimeria bovis were obtained in large numbers from mature schizonts, after concentration of these by repeated washing and sedimentation. The appearance of living merozoites, as well as their flexing and gliding movements, were described with the use of the phasecontrast microscope. In specimens stained with protargol the anterior portion of the body was found to have a cap-like covering with a terminal pore, and a median rod-like structure. Prominent granules occurred in the posterior 2/3 of the body, with one granule characteristically located at the posterior extremity. The nucleus was in the posterior 1/3 of the body. In Feulgen and acridine orange preparations the chromatin was arranged as a ring at the periphery of the nucleus; at irregular intervals there were coarse clumps, usually 3 to 5 in number. Numerous small glycogen granules were present in the posterior 2/3 of the body. No sudanophilic lipids were demonstrated. The entire body of each merozoite showed a diffuse positive reaction with the ninhydrin method. These merozoites were found to be similar in certain morphological features to Toxoplasma gondii.

Nitrofurazone as a prophylactic agent against experimental bovine coccidiosis.

In 3 experiments, each with 12 calves about 2 months old, the administration of nitrofurazone in the feed at 5.0 or 7.5 mg/kg of body weight daily for 6 weeks, beginning 4 days before inoculation of 50,000 to 100,000 Eimeria bovis occysts, did not prevent the occurrence of coccidiosis. In each of these experiments the calves were allotted to 3 groups, each including 2

inoculated calves housed in the same pen with 2 uninoculated calves. Little or no infection was observed in the uninoculated calves. In 2 of the 3 experiments, sporulation of oocysts apparently did not occur in the pens during the course of the experiments because of low temperatures; in the 3rd experiment the calves evidently had some degree of immunity as a result of natural infections. The weight gains of the treated calves were not consistently different from those of the untreated calves, but the calves given 7.5 mg/kg made smaller average weight gains than those given 5.0 mg/kg. The results of 1 experiment with 8 calves, 2 months old, indicated that nitrofurazone in the feed at 10 mg/kg for 3 weeks beginning 1 week before inoculation of 100,000 oocysts each of E. bovis and Eimeria zurnii, had only questionable value in preventing coccidiosis. In 1 experiment with 6 calves about 4 months old, nitrofurazone administered in gelatin capsules at 30.0 mg/kg for 4 days starting 15 days after inoculation of 100,000 E. bovis oocysts, was effective in controlling coccidiosis.

Amprolium for control of coccidiosis in calves. Sixty-nine calves were used in 6 experiments to determine the efficacy of amprolium in controlling Eimeria bovis infections. In one of these experiments, 3 additional calves were treated with ethopabate. In each experiment three or four groups each of 3 calves about 2 weeks old, were inoculated with 50,000 or 100,000 occysts; two or three of these groups were given liquid amprolium in the milk. In each of three experiments, one group of 3 calves was left uninoculated until 30 days after the original inoculation, then all groups were challenged with 1 million occysts. In five experiments, treatment at 16.25 mg/lb for 21 days, beginning on the day of inoculation, provided good to excellent control of coccidiosis, as did such treatment at 65 mg/lb and 10 mg/lb in two experiments.

In four experiments, calves treated at 65 mg/lb for 5 days beginning 13 days after inoculation, had less severe signs of coccidiosis and discharged fewer oocysts than untreated calves, but the results of this treatment were not as good as those of the 21-day treatments. In one experiment, treatment with amprolium at 65 mg/lb or ethopabate at 1 gram/lb for one day, 13 days after inoculation, had little or no effect on the infections. Calves which had been treated had less severe infections after challenge than did controls not previously inoculated. (Logan, Utah) (ADP b1-29)

H. <u>Investigations of Anaplasmosis</u>

Research at the Beltsville Parasitological Laboratory has shown that a partially purified antigenic protein has been isolated from red blood cell hemolysates obtained from cattle with acute anaplasmosis. Concentration, characterization and immunogenic studies on the material are under way.

Thin-section studies on the ultra-structure of \underline{A} . $\underline{\text{marginale}}$ have revealed that the parasite contains, in the bovine red blood cell, from one, to at least six, smaller organized units or bodies. The detailed structure of these sub-units has not been clearly demonstrated. A mild type of

A. marginale, naturally occurring in the United States, has been observed to be of similar pathogenicity to A. centrale, the so-called vaccine type used in Africa and elsewhere for premunition.

Field tests are in progress to determine if a "dead Anaplasma" antigen will protect cattle against natural exposure in a degree sufficient to prevent economic losses from the disease. (Beltsville, Md.) (ADP bl-30)

I. Histochemistry of Gastro-Intestinal Nematodes of Cattle

Research work at the Division's Regional Animal Disease Laboratory at Auburn, Alabama, was reported as follows:

Studies conducted on the histochemistry of the host response to the presence of larval nodular worms, <u>Oesophagostomum radiatum</u>, in the wall of the small intestine of the calf have demonstrated that the presence of these larvae is associated with a decrease in the connective tissue protein collagen and an increase, real or apparent, in glycoprotein around the site of the larval worms. These alterations in the chemistry of the tissue of the calf appear early in the infection and disappear as healing is completed.

The presence of the larval worms is also associated with an increase in the activity or amount of the enzymes alkaline phosphatase, acid phosphatase, and non-specific esterase in the vicinity of the lesion. The increase in these enzymes is apparently associated with metabolic changes in the cells of the host as they respond to the presence of the parasites. Infection by the young nodular worms produced no effect on the distribution and abundance of the enzyme leucine aminopeptidase during the stages of the infection studied.

Ketoenolic lipoids, originally discovered in certain other animals by the Japanese scientist Yukio Hamazaki, were found for the first time in the tissue of cattle and nematodes. The amount of these chemicals is increased around young nodular worms in the wall of the small intestine during some, but not all stages of nodular worm disease in cattle. These same chemicals also occur in the intestines of young nodular worms.

(Auburn, Alabama) (ADP bl-32)

J. Parasites of Cattle with Emphasis on Stephanofilarial Species

Studies made at the Division's Regional Animal Disease Laboratory at University Park, New Mexico, have shown that Stephanofilaria stilesi is a worm parasite which causes extensive sores on the skin of cattle. This chronic condition is called "stephanofilarial dermatitis." It is widespread in the United States and other parts of the world and is of much economic importance. Research based on natural and experimental infections at the University Park Field Station proved that the disease is transmitted by horn flies. Effective medicinal treatment directed at the worms in the

lesions is not yet available. Perhaps diligent control of horn flies would result in a lower incidence of the disease.

(University Park, New Mexico) (ADP bl-33)

K. Effect of Stocking Rate and Rotational Grazing on Internal Parasitism in Beef Cattle

This work was done at Experiment, Georgia, under the auspices of the Division's Regional Animal Disease Laboratory at Auburn, Alabama. The report shows that experiments suggest that rotational grazing results in increased rate of stocking of the pastures and consequently increases parasitism in beef cattle. Two lots of winter temporary pasture were stocked at the same time - one was grazed continuously and the other was grazed on a four-way rotational system. A third lot was also rotationally grazed, but the stocking rate varied with the carrying capacity of the pastures. The steers from the two rotationally grazed groups had more worms and made a lower average daily gain. The steers grazed rotationally where the stocking rate varied had the greatest number of worms. Although various factors may be responsible for an increase in parasitism, the increased stocking rate is very likely the most significant factor.

(Experiment, Georgia) (ADP b1-34)

L. Under a PL 480 grant to the School of Veterinary Medicine, University of San Marcos, Lima, Peru, research is being conducted on Environmental Factors Influencing Parasites and Parasitic Diseases of Economical Importance in Ruminants (cattle, sheep, alpacas). This project was reviewed during 1964 by a Department scientist who visited the Principal Investigator in Peru. The reviewer explained the use of several methods adaptable under conditions in Peru for improvement of the investigations.

The accomplishments for the third year of research are 1) collection of climatic data in relation with the seasonal incidence of parasitic diseases of livestock; 2) preparation of a calendar of treatment and control of parasitic diseases of livestock, according with the management and seasonal occurrences of these diseases; 3) a check list of parasites identified in our laboratory of parasitological research, during the period 1961 to 1964; 4) bionomic of fresh water snails, transmissors of Fasciola hepatica, and 5) a pamphlet on Parasites and Parasitic Diseases of Lamb pacos (Alpacas) in Peru. (PL 480) (S8-ADP-1)

Diagnosis and Methods of Prevention and Treatment of Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle and Further Characterization of the Causative Agents. These investigations were conducted under a PL 480 grant to the School of Veterinary Parasitic Diseases, Montevideo, Uruguay. The work involved studies on 1) Tick development in vitro - Results - Nymph stage was achieved. Nymphs were kept alive for a period of 45-50 days in vitro. 2) Tissue cultures from bovine infected with Babesia bigemina - Results - Dermal tissue, spleen and brain were studied. 3) Tissue culture from bovine infected with Anaplasma marginale and uninfected bovine -

Results - Primary cultures showed several macrophages with parasited red cells. 4) Electrophoretic studies of bovine serum - Results - In the group inoculated with Anaplasma centrale serum protein variations occurred. The variations in general appeared in irregular form from one animal to another, differently to what occurs in anaplasmosis by A. marginale. 5) Electrophoretic studies on bovine serum - Results - Serum protein of cows infected with A. centrale and challenged with A. marginale were studied in 22 animals: a) pre-patent period - during this period only a decrease of alpha-globulin value was observed, b) patent period - at the beginning of this period, value of beta-globulin increased - remained until incidence of A. marginale, then returned to pre-inoculation value, gamma-globulin and total proteins decreased, and alpha-globulin in this period returned to pre-inoculation value. c) when red blood cells decreased under one percent; total serum protein and gamma increased, while albumin alpha and beta-globulin remained at pre-inoculation levels. d) no significant variations occurred at serum protein levels in the control cows free from A. centrale and A. marginale. (PL 480 Uruguay)

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